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THE EFFECTS OF PHOSPHORUS ENRICHMENT ON THE DOMINANT PHYTOPLANKTON COMMUNITIES OF CHANEY LAKE

A Thesis

Presented to

The Faculty of the Department of Biology

Western Kentucky University

Bowling Green, Kentucky

In Partial Fulfillment

of the requirements for the Degree

Master of Science

By

Kathy A. Tehrani

August 1999

THE EFFECTS OF PHOSPHORUS ENRICHMENT ON THE DOMINANT PHYTOPLANKTON COMMUNITIES OF CHANEY LAKE

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THE EFFECTS OF PHOSPHORUS ENRICHMENT ON THE DOMINANT PHYTOPLANKTON COMMUNITIES OF CHANEY LAKE

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THE EFFECTS OF PHOSPHORUS ENRICHMENT ON THE DOMINANT PHYTOPLANKTON COMMUNITIES OF CHANEY LAKE

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Directed by: Jeffrey D. Jack, Gary E. Dillard, Douglas M. McElroy

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Chaney Lake is a temporary karst wetland located in southern Warren County, Kentucky. Because of an impermeable chert layer between the surface and the porous limestone, Chaney Lake fills with water over the winter and spring and then gradually drains over the summer. Three experiments were conducted over the course of the 1998 flooding season to assess the effects of phosphorus addition on the phytoplankton community in Chaney Lake. Ten plastic-sided mesocosms were constructed and placed in the marsh area of the wetland at three different periods: early spring, early summer, and late summer. Phosphorus in the form of K_2HPO_4 was added by volume to five of the enclosures, with five remaining as controls. The water chemistry parameters measured included soluble reactive phosphate, nitrates, ammonia, and ammonium. Physical parameters such as depth, temperature, dissolved oxygen, dissolved oxygen %, specific conductivity, pH, and turbidity were measured. Chlorophyll *a* and phytoplankton community structure were also assessed for all three of the experiments. The various parameters were analyzed with Discriminate Function Analysis, ANOVAs, and Pearson Correlations to determine whether there were significant differences between

the treated and control enclosures. The results indicate significant differences ($P<0.05$) between the treated and control enclosures with respect to nitrates, turbidity, and ammonia. There were no significant differences in SRP, chlorophyll *a*, DO, DO%, SPC, pH, temperature, depth, and phytoplankton composition. It was concluded that phytoplankton in Chaney Lake may not be limited by phosphorus. However, phosphorus may limit bacterioplankton, periphyton, or both. The high nutrient concentrations with low elemental ratios indicate that bacterioplankton may be more efficient in phosphorus utilization and possibly play an important role in nutrient availability for phytoplankton in the marsh area of Chaney Lake.

INTRODUCTION

A wetland is defined as an ecosystem that depends on constant or recurrent shallow inundation or saturation at or near the surface of the substrate, and on the presence of physical, chemical, and biological features reflective of such conditions, specifically, hydric soils and hydrophytic vegetation. Defining wetlands has been difficult, especially for legal purposes, because they have a considerable range of hydrologic conditions and because they vary in size, location, and human influence (Mitsch 1993). The most widely accepted definition of wetlands was adopted by wetland scientists in the U.S. Fish and Wildlife Service in 1979 (Cowardin et al. 1979).

> "Wetlands are lands transitional between terrestrial and aquatic systems where the water table is usually at or near the surface or the land is covered by shallow water... Wetlands must have one or more of the following three attributes: (1) at least periodically, the land supports predominantly hydrophytes, (2) the substrate is predominantly undrained hydric soil, and (3) the substrate is nonsoil and is saturated with water or covered by shallow water at some time during the growing season of each year." (Cowardin et al. 1979).

There are some important characteristics that can be used to identify wetland ecosystems and distinguish them from other ecosystems. Three of these characteristics are water, substrate, and the biota.

Wetlands are on the interface for the major water reservoirs in the hydrologic cycle: surface water, groundwater, atmospheric water, and in some places, sea water. Standing water in wetlands occurs as a result of surface flooding or when the water table rises above the saturated zone. Wetlands can exist where the surface is flooded for extended

periods or where there is increased soil saturation as a result of ground water moving or standing close to the land surface (Lewis 1995). The duration and frequency of saturation in a site may vary according to the hydrogeologic setting and on regional differences in physiography, climate, and past moisture conditions. Hydrological conditions affect many abiotic factors including soil anaerobiosis, nutrient availability, and, in the cases of coastal wetlands, salinity. Hydrology affects the species composition and richness, primary productivity, organic accumulation, and nutrient cycling in wetlands. The biotic components, in return, are active in altering the wetland hydrology. For example, evapotranspiration, the moisture that passes through vascular plants combined with the water that vaporizes from water or soil, plays an important role in wetland ecology (Gosselink and Turner 1978). The duration of saturation can be depicted from a wetland's hydroperiod, (hydrologic signature of a wetland) on a graph that shows the position of the water table in the area over time. A wetland's hydroperiod integrates all aspects of its water budget, rainfall, evapotranspiration, runoff from adjacent areas, flooding, and net seepage of groundwater (Lewis 1995). Hydrology controls the abiotic and biotic characteristics of wetlands. Abiotic characteristics such as soil color, soil texture, and water quality depend on the distribution and movement of water, as do the abundance, diversity, and productivity of plants, vertebrates, invertebrates, and microbes. There are three major causes of hydrological variation between wetland ecosystems. First is geographic setting, for example, whether the wetland is located in a floodplain, estuary fringe, or slope. The second is the water source, whether the wetland is fed primarily by precipitation, groundwater, or riverflow. The last major cause of hydrological variation is the hydrodynamics of the wetland, such as unidirectional flow

or reversing flow. For example, tidal and brackish marshes and mangrove wetlands exhibit reversing flow, while marshes and swamps fed primarily by incoming streams exhibit unidirectional flow. Depressional wetlands are maintained by overland flow, groundwater, and precipitation rather than channelized flow. Riparian wetlands show seasonal or periodic pulses of water level that are delivered from overbank flow carrying nutrients and organic matter. Estuarine fringe wetlands are pulsed hydrologically by daily tides. Slope wetlands, such as the seeps that occur where groundwater reaches the surface, are maintained by relatively constant sources of water. Peatlands can be maintained entirely by precipitation (Lewis 1995). There are other important factors which affect the ecology of wetlands such as organic matter accumulation, natural disturbances, floods, fire, or herbivory. In addition, nutrient transformation, such as the input of nitrogen and phosphorus by precipitation, overbank flow from streams, lunar tides, movement of surface and groundwater, and biological fixation of nitrogen play a role in wetland ecology (Lewis, 1995).

Wetland soil is the medium in which many of the wetland chemical transformations take place and the primary storage area of the chemicals needed by most wetland plants. Wetland soils are described as hydric, meaning the soil is saturated, flooded, or ponded long enough during the growing season to develop anaerobic conditions in the upper part (US Soil Conservation Service 1987). There are two types of wetland soils: mineral soils and organic soils. Mineral soils have less than 20 to 35 percent organic matter, neutral pH, high bulk density, low porosity, high hydraulic conductivity, low water holding capacity, and generally high nutrient availability. They are typical of riparian forest wetlands and some marshes. Organic soils have greater than 20 to 35 percent organic

content, acidic pH, low bulk density, high porosity, low to high hydraulic conductivity, high water holding capacity and low nutrient availability and are common in northern peatland wetlands (US Soil Conservation Service 1975).

The vegetation of wetlands is distinctive primarily because flooding and soil saturation create conditions that most plants cannot tolerate. Saturation of soil with water effectively blocks the entry of oxygen from the atmosphere. Oxygen that enters the soil is readily depleted through plant roots and microbial populations. Lack of oxygen in the roots is a source of stress for plants that lack special adaptations to bring oxygen to the roots from above or to function without oxygen in the root zone. After oxygen is consumed in the soil, some microorganisms can use other soil oxidants, such as nitrate and oxidized manganese and iron compounds to carry on their metabolism. Under some conditions, this anaerobic microbial activity can produce toxic substances that add to the oxygen deficiency stress. Also, if large amounts of organic matter are present, anaerobic bacteria can convert sulfate and organic sulfur compounds to hydrogen sulfide and other reduced-sulfur compounds that are especially toxic to plants. Organic acids and other reduced-organic compounds that are produced from microbial activity also can be toxic to plants. Plants that grow in anaerobic soils must have special adaptations that allow absorption of nutrients and water without absorption of toxins. The greater the redox potential of the soil, the more severe the stress on plants. Stress ranges from moderate, if created by the absence of oxygen, to more severe, if created by the absence of oxygen and the presence of various toxic substances. Anoxia is not the only factor that can produce distinctive wetland vegetation. Plants that have exceptionally high requirements for water can be restricted to wetlands (Lewis 1995).

Wetlands include some unique functions that add to their importance as separate ecosystems. Functions of wetlands can be defined broadly as all processes and manifestations that occur in wetlands. Some of these functions are shared by uplands; however, some are unique to wetlands, such as maintenance of breeding habitat for some aquatic bird species. Because most wetlands are adjacent to surface waters, they have a direct impact on water quality through filtering and transformation capacity. Seasonal wetlands retain their capacity for storage of surface water although they are dry during some of the season. Individual wetlands also interact with adjacent landscape and with other wetlands, which is critical to the support of many organisms and contributes to biodiversity (Lewis 1995). Another unique function of wetlands is their ability to process dissolved and suspended material from an area much larger than their own, which explains their influence on water quality. This function is due to accumulating nutrients, trapping sediments, and transforming a variety of substances.

The presence of a dry period separates wetlands into the functional categories of seasonal and permanent wetlands. Bogs and fens are permanently inundated, but seasonal wetlands are characterized by a dry period when all or most of the area reverts to terrestrial status. Most of the organic matter produced during the flooding season is decomposed in the well-oxygenated dry season. Algae, aquatic plants, insect larvae, and other small organisms that can survive the rigorous environmental fluctuations often become superabundant. Migratory birds that breed in seasonal wetlands feed on the dense but short-lived explosion of the insect population (Horne 1994).

Algae are fundamental components of the biological, chemical, and physical processes of wetland ecosystems. Most obvious is their role as primary producers and their place in

food webs. Algae contribute to wetland nutrient cycles as sources of dissolved organic matter, nitrogen, and short-term sinks for phosphorus. In addition, algae play an important role in wetland detrital food web, as producers of organic matter, consumers of organic substrates, and facilitators of microbial decomposition (Reynolds 1986).

Natural phytoplankton communities are composed of populations of species. For these species to persist through time, their cellular growth rate (K′) must exceed or equal losses to dilution (K_w), sedimentation (K_s), physiological death (K_d), and grazing (K_g), therefore, $dN/dt = K'N - K_wN - K_sN - K_dN - K_gN$ and $N_t = N_0\exp(K' - K_w - K_s - K_d - K_g)t$, where dN/dt is population growth, N the abundance of a phytoplankton species, N_t the population biomass at time t, and N_0 the biomass at time zero. N and dN/dt are determined by the difference between K′ and the sum of the loss terms. An increase in abundance can be generated by increasing K′ or decreasing one or more of the loss terms. Consequently, any species can be limited by its growth coefficient, its loss terms, or both. It should be noted that a population increases at both low and high K′; therefore a low abundance does not mean that K′ is limited in any way (Hecky and Kilham 1988, Reynolds 1984). Nutrients affect this equation directly through K′, which is a function of light, temperature, and nutrient supply.

Phytoplankton can be limited by the availability of nutrients when light and temperature are adequate and loss rates are not excessive. Laboratory cultural studies have established that internal cellular concentrations of nutrients determine phytoplankton growth rates, and these studies have shown that it is often difficult to relate growth rates to external concentrations, especially in natural situations (Hecky and Kilham 1988). In order to reproduce, algal cells require elements in relatively fixed

proportions known as the Redfield ratio. Despite differences among species, it is possible to consider a mean nutrient composition for algal cells growing without nutrient limitation. A relatively narrow range of elemental composition has presumably evolved because all algal cells have to perform similar metabolic functions and have qualitatively similar structural requirements (Hecky and Kilham 1988). Deviations from mean dissolved nutrient concentrations are common in both freshwater and marine ecosystems. In marine and freshwater systems, biologically active elements are constantly being removed from surface waters by nutrient uptake and sedimentation. There are other biogeochemical processes, such as denitrification, nitrogen fixation, phosphorus adsorption, and cultural eutrophication that can selectively remove or enrich nutrients. On average, in freshwater situations, P is the most likely of the macronutrients to become limiting to algal growth (Hecky and Kilham 1988).

Phytoplankton communities are complex mixtures of species with highly individualistic life-history characteristics, especially in regard to meeting their nutrient requirements. Nutrient concentration data can be used to calculate fluxes of nutrients in aquatic ecosystems. If algal growth can be shown to be more dependent on one nutrient flux then any other, than that nutrient may limit algal growth. Nutrient-limited growth can be modeled using either the Droop model or the Monod model. The Droop model of phytoplankton growth relates growth rates to internal nutrient concentrations (Hecky and Kilham 1988). This model is considered to provide a reasonable representation of nutrient-limited algal growth. The Monod model relates phytoplankton growth to external concentrations of dissolved nutrients (Hecky and Kilham 1988). In studies of lakes in Canada, Schindler (1977) found that mean annual chlorophyll concentrations

were statistically dependent on the mean annual P concentrations regardless of N:P loading rate of the lake. He concluded that P ultimately limits phytoplankton growth in lakes because these ecosystems can draw on atmospheric sources of C and N to meet their requirements (Schindler 1977). The amounts of nitrogen in the water also can have an effect on phosphorus and phytoplankton growth. Elser et al. (1990) found that considerably larger growth responses occurred when nitrogen and phosphorus were added together, indicating that, while instantaneous growth may have been more limited by one nutrient or the other, total algal biomass production was commonly limited by the availability of both N and P. In several whole lake studies it was concluded that both P and N were potentially limiting to algal growth and considerable interaction occurred when N and P were added in combination; however, P-reduction will generally be a more reliable means to achieve lower algal standing stocks in the long term (Elser et al. 1990; Levine et al. 1997).

Phosphorus is a common growth-limiting factor for phytoplankton in lakes because it is often present in low concentrations. Living matter contains about 0.3 percent dry weight phosphorus. Phosphorus plays a role in the structural link in the genetic materials of DNA and RNA, and in ATP, phosphorus is involved as short-term energy of biochemical reactions. Additionally, it is a component in the phospholipid membranes of cells. There are four reasons for the limitation of phosphorus in freshwater ecosystems:

1) Rock breakdown in the watershed releases phosphorus that is not readily available for biological breakdown in streams and lakes because it is in the form of inorganic mineral phosphorus.

2) The root zone on land intercepts and retains most soluble P compounds.

3) Since there is no gaseous phase in the phosphorus cycle, rainwater contains little phosphorus.

4) Any soluble PO_4 released into water is rapidly adsorbed onto particles or precipitated with other compounds and is not readily available for algae (Horne 1994).

Inorganic phosphorus makes up 0.1 percent of continental rocks. Since plankton typically requires N:P ratio of 7:1 by weight or 16:1 by element (known as the Redfield ratio) phosphorus depletion is likely to occur in many freshwater systems (Redfield 1958), especially where nitrate levels are high (> 100 g/liter). The validity of this ratio concept is shown by the comparison of the proportion in which the elements exist in the plankton and the proportions in which they vary in samples of water from the open sea. The analysis of many samples of plankton, taken in a variety of places with nets designed to take organisms of different sizes indicates that atoms of phosphorus, nitrogen, and carbon are present on the average in ratios: 1:16:106 (Redfield 1958). In general, if the ratio of N:P > 10 (by weight), phosphorus is considered to limit phytoplankton growth (Horne 1994). PO_4 is supplied to freshwater ecosystems through the erosion of PO_4-rich particles and clays, sewage and agricultural run-off. Normally several forms of phosphorus are measured in lakes and rivers. For unfiltered water, two forms are analyzed: *total reactive phosphorus* (TRP), the PO_4 that reacts with molybdate, and *total phosphorus* (TP), which reacts in phosphorus fractions present, including any released from soluble and particulate phosphorus compounds after perchloric acid digestion. In filtered water, PO_4 is measured by *total soluble phosphate* (TSP; also *total dissolved phosphate*, TDP), which is the dissolved PO_4 that reacts with molybdate, this phosphate analysis sometimes includes colloidal material in the water. Another form of

phosphorous most commonly measured in freshwater ecosystems is *soluble reactive phosphorus* or SRP. SRP is the reactive phosphorus most commonly used by primary producers. SRP and TRP both correlate well with biologically available phosphorus as measured by the growth of algae (Horne 1994).

Algae have evolved three methods to overcome phosphorus deficiencies in the water column: 1) luxury consumption; 2) the ability to use phosphate at low levels (low K_m); and 3) alkaline phosphatase production.

In luxury consumption more phosphate is taken up by the phytoplankton than required for growth and is stored in cellular structures called polyphosphate granules. In most lakes the phosphatase growth constant K_m is very low for natural phytoplankton, around 1 – 3 (g/liter PO_4 –P), which means the enzyme system in algae is not saturated for much of the time under natural conditions. There may be considerable variation in K_m between algal species, and as available phosphate decreases this lack of phosphate could play a role in species succession (Horne 1994). Because phosphate is recycled rapidly, the rate of phosphate uptake (V_{max}) is also very important. A higher uptake rate can thus compensate for the lack of a mechanism to remove phosphate at very low levels (Horne 1994). Alkaline phosphatase is an esterase that cleaves the bond between phosphate and an organic molecule to which the phosphate is attached. This reaction results in free phosphate available for plant growth. The production of alkaline phosphatase by algae is an important adaptation to an environment low in soluble reactive phosphate availability but high in other phosphorylated compounds (Horne 1994).

Zooplankton, fish, macrophytes, algal decomposition, and bacterioplankton all play major roles in the amount of phosphorus available for phytoplankton use. Zooplankton

and fish excrete nutrients directly into the water, where they are available for phytoplankton growth. Studies have shown that fish can alter P cycling both directly through excretion/egestion and indirectly through effects on zooplankton excretion. Fish can thus have a strong impact on phytoplankton community structure and biomass (Vanni 1990). Zooplankton excrete approximately 10 percent of their body phosphorus daily, roughly half mineral phosphate and half organic phosphate. Therefore, changes in the phytoplankton composition of freshwater ecosystems can be related to changes in zooplankton numbers (Corner and Davis 1971). Many studies have shown the direct effect of bacterioplankton on the amount of phosphorus available for phytoplankton consumption. Most lake environmental studies indicate that bacteria sequester most available phosphate at ambient concentrations (Cotner and Wetzel 1992). Both phytoplankton and bacteria obtain nutrients from the dissolved phase, but this does not necessarily mean that their individual nutrient budgets are similar. Storage ability and the temporal pattern of supply relative to growth are not identical for bacteria and algae, meaning one may rely on storage more than the other (Sterner et al. 1995;Tarapchak et al. 1990;Currie 1990; Vadstein et al. 1988).

In addition to biological factors, several studies have shown that freshwater wetland soils and vegetation can also affect the P levels of the water. Wetland soils can function as either sink or source for P to the overlying floodwater moving through the wetland. Physical, chemical, and biological processes functioning in overlying water and underlying sediments regulate P dynamics in wetlands. A significant portion of floodwater and pore water P can be removed through uptake by macrophytes and algae (Gale et al. 1994). Rooted macrophytes normally obtain about 85 percent of their

phosphorus by absorption of phosphate directly from the interstitial soil pore water. Over the year this phosphorus is released to the water by the decay of freshwater macrophytes (McRoy et al. 1972). Like macrophytes, phytoplankton and attached algae release phosphorus when they decay.

Many kinds of wetland ecosystems are found within the United States. These range from small, discrete sites such as Thoreau's Bog in Massachusetts or Four Holes Swamp in South Carolina to large, spatially complex ones such as the Great Dismal Swamp in Virginia and North Carolina or the peatlands of northern Minnesota. In very large wetlands, such as extensive peatlands, marshlands, bottomlands, and river floodplains, internal spatial variation can be great. Examples include the Great Dismal Swamp, which consists of at least four major wetland plant communities integrated with lakes and streams; the Everglades, which includes sloughs, sawgrass prairies, and wet shrub islands; the Mississippi delta, which has swamps, marshes, lakes, and rivers; and the peatlands of northern Minnesota, which includes bogs, marshes, and lakes. In these areas, the gain, loss, and transformation of elements takes on continental or biospheric proportions (Lewis, 1995).

In addition to biological and chemical influences, wetlands are affected by their hydrological setting. For example, karst wetlands are greatly influenced by their geological properties. Most karst areas are formed on carbonate rocks such as limestone or dolomites-sometimes on gypsum. Limestone karst is the most extensively developed, has the broadest regional extent, and has the most elaborate and highly integrated underground drainage and cavern systems (White 1989). Karst landscapes are often pitted lands of sinkholes, limestone towers and steep-sided hills, underground drainage,

and caves. Karst landscapes have three unique characteristics that affect wetland formation:

1) The soil-bedrock contact is often irregular. There are deep solution cavities along joints and fractures which can be completely masked by soil cover.

2) The network of solution cavities extending downward from the surface permits the efficient transport of clastic material by groundwater infiltration. The phenomenon of soil piping is extremely efficient in transporting soils from the surface to the subsurface.

3) The presence of shallow solution cavities makes subsidence and bedrock collapse a very real possibility (White 1989).

The most characteristic feature of karst terrain is the concentration of water flow in underground solution conduits. Since much of the input to the karst groundwater system is through sinkholes and sinking streams, and because of the open character of the aquifer and lack of soil cover, karst systems are susceptible to pollution. Sources of pollution include industrial and hydrocarbon wastes, nitrates, herbicide and pesticide residue, highway spills, and leaking sewer lines, pipelines, and storage tanks (White 1989). When pollutants make their way into an open conduit, they can be transmitted for very long distances with relatively little dilution and very little dispersion (White 1989).

In the south-central region of the United States karst landscapes cover large areas in Kentucky, Tennessee, and Indiana. The south-central karst landscapes have geologically developed on Mississippian limestones. The main aspect in shaping this landscape is the dissolution of the limestone bedrock by CO_2-rich groundwater, resulting in caves, underground rivers, sinking streams and sinkholes. Therefore, there little surface water in karst areas, and distinguishing between surface and groundwater can be difficult.

In areas that are not karst in hydrology groundwater can take a long time to receive surface water, and flow rates can be measured in centimeters per day. However, in karst areas the rate that groundwater receives surface water is much faster. For example, surface flowing streams can retreat underground and flow for some distance, then re-emerge and flow on the surface again. These underground streams can flow as fast as surface streams, thus allowing groundwater move several miles in a short period of time.

Ephemeral lakes were first described by Cvijic (1893) and referred to as karst poljes. Karst poljes appear to be broad, closed depressions or sinkholes that have diverse hydrologic conditions. They may be occasionally or seasonally flooded, or sometimes contain perennial water bodies (Bögli 1980). There are three water conduits to and from the surface of these lakes: springs, swallets and estavelles. In springs, water flows onto the surface from underground. Swallets are places where the water flows underground; estavelles are places that water can flow both in and out depending upon the level of groundwater (Milanovic 1981). These conduits are connected to the underground cave network, providing the water that fills and drains ephemeral lakes. During the wet season as groundwater levels rise out of the sinkholes, lakes are formed. With the return of drier conditions, groundwater levels decrease and water drains back through the estavelles where the water initially originated.

Chaney Lake State Nature Preserve (elev. 177.2m; lat. 36° 53' 05'' N; long. 86° 25' 16''W) has been owned by the Commonwealth of Kentucky since 1991 and is managed by the Kentucky State Nature Preserves Commission. Chaney Lake is 89 hectares in area and is a seasonal karst wetland located in southern Warren County, Kentucky, approximately 300 meters west of US Highway 31W. Found within the headwater area

of the large (>100 km^2) Lost River Groundwater Drainage Basin, this lake forms in broad, shallow, closed depressions that overlie cave passages carrying underground rivers. Chaney Lake is usually flooded between the months of January and August. Water from Chaney flows underground before emerging again in Rich Pond, another ephemeral karst lake approximately 1.5 km to the east. Water from Rich Pond flows through the cave network in a northernly direction several kilometers before emerging and draining into the Barren River (Crawford et al. 1987, Groves and Crawford 1991). Before State ownership, large portions of the lake had been cleared and cultivated for agriculture after the water in the lake drained. Additionally, there was an unsuccessful attempt at draining the lake via a ditch. However, during heavy rains this ditch would act as an intermittent stream and actually feed water into the lake. Other human impacts on the lake include powerlines, corn and soybean fields surrounding the lake, and pastures for cows, horses and ostriches in the area that directly drains into the lake.

Chaney Lake and nearby Rich Pond as well are perched on thin chert layers separating the lake from the underlying cave system (Crawford et al. 1987, Groves and Crawford 1991). The lakes fill and drain via estavelles that have formed through fissures in the chert layer. During the relatively wet winter and spring seasons, the cave's capacity to transmit flow increases, causing the water level to rise and fill the floor of these depressions through estavelles. Following high levels of groundwater discharge, water spreads into several pools, and this impermeable chert layer allows the wetland to hold water for an extended amount of time. During this period, Chaney Lake can expand to over 1.5 km in diameter. Consequently, as drier conditions return in summer, the lake drains back into the cave system. Chaney Lake is one of the most important sites for

migrating waterfowl and shorebirds in Kentucky and is used extensively by many nesting song birds. The mature hardwood trees surrounding the open marsh habitats consists mainly of *Acer rubrum*, *Fraxinus pennsylanica*, *Liquidambar styraciflua*, *Quercus bicolor*, and *Quercus palustris* (personal communication Kentucky State Nature Preserves Commission). The preserve is surrounded by agricultural farmland, which must influence the chemical and physical composition of the wetland.

The present was study focused on identification of the dominant phytoplankton communities and observation of the effects of phosphorus enrichment in Chaney Lake during the 1998 flooding season. Plankton are limited by phosphorus because of the 16:1 elemental N:P ratio required for plankton growth, known as the Redfield ratio (Redfield 1958). In P-rich environments there is low N:P, and competition is for nitrogen along the N:P gradient. However, in critical phosphorus or low P environments there is a high N:P, and competition occurs for available phosphorus independent of nitrogen concentrations. At times nitrogen may limit phytoplankton production in temperate eutrophic lakes, especially where phosphate concentrations are relatively high. The interaction between the total amounts of nutrient and their partitioning between the various populations will bias community structure in a particular direction; this can be observed through N:P quotas (Hecky and Kilham 1988).

The hypothesis was that phosphorus enrichment does change the phytoplankton communities of the lake and, subsequently, the availability of other nutrients. A traditional experimental enrichment approach designed to observe the P-levels of the marsh area of Chaney Lake through the 1998 flooding season was undertaken to

determine the effects of phosphorus enrichment on the dominant phytoplankton communities.

METHODS AND MATERIALS

Seven different habitats in and around Chaney Lake, based mostly on the dominant vegetation, can be identified:

Location 1: An open brushy field on the north side of the lake having been previously cleared for agriculture.

Location 2: A stand of mature hardwood trees directly adjacent to and south of *Location 1*. This area has not been cleared recently and consists mainly of *Liquidambar styraciflua*, *Quercus palustris*, *Quercus lyrata*, *Quercus bicolor*, *Acer rubrum*, and *Platanus occidentalis* (personal communication Kentucky State Nature Preserves Commission).

Location 3: Immediately adjacent to and south of *Location 2*. This area has been cleared in the past and is now dominated by *Liquidambar*, *Fraxinus*, and *Quercus* saplings.

Location 4: A wooded area that borders the open marsh The dominant tree species here is *Salix nigra*, other species include *Acer rubrum*, *Quercus bicolor*, *Populus* species and *Platanus occidentalis* (personal communication Kentucky State Nature Preserves Commission).

Location 5: The open marsh, approximately 250-400 m^2 in area is dominated by aquatic vegetation such as *Polygonum pensylvanicum*, *Cephalanthus occidentalis*, and *Proserpinaca palustris* (personal communication John Andersland) .

Location 6: An intermittent and shallow stream that flows into the lake from the south side. The stream flows through several fields and a logging site adjacent to the preserve and eventually terminates in the preserve. This stream and nearby areas receive heavy deposition of silt from the fields and logging site.

Location 7: A small area on the south border of the lake that contains several estevelles clustered together. During rainfall events water can be seen flowing from small holes in the ground.

The study was conducted at *Location 5* (open marsh) of Chaney Lake during the 1998 flooding season. The open marsh was chosen because it holds water longer than any other habitat within Chaney Lake. The average depth of the water is approximately 35-45 cm. Ten enclosures, each $1m^3$ were constructed with ½ inch PVC piping. Plastic was wrapped around the sides of the enclosures and tied at the corners. There were five replicates of each treated and non-treated mesocosms. After approximately a week of pre-treatment and equilibration, phosphorus in the form of K_2HPO_4 was added to five randomly chosen corrals. The amount of phosphorus enrichment was calculated by multiplying the highest level of the soluble reactive phosphate levels from the previous year with the volume of water in each corral, approximately 0.4 mg/l were added to the treated enclosures for the three experiments. Three separate experiments in early spring, early summer, and late summer were conducted and the sampling was done weekly for five weeks, except for the last experiment which had to be concluded after three weeks as a result of water draining out of the marsh. Water samples for nutrient chemistry were taken in 250ml sterile polyethylene bottles and for chlorophyll analysis in 1l sterile polyethylene bottles. Phytoplankton was collected by pouring approximately 12l of water through a phytoplankton net and then allowing the organisms to concentrate at the bottom. Whole water samples were taken so smaller algae would not be lost and all organisms were retained. Depth and physical parameters such as temperature, specific conductivity, dissolved oxygen percent, dissolved oxygen, pH, and turbidity were

measured on site with a Water Quality Monitor Instrument (Yellow Springs Incorporated). The water samples for chlorophyll were taken back to the lab and subsamples were filtered through 0.47 μm Whatman GF/F filters. The filters were stored in plastic petri dishes and frozen at -20°C for later fluorometric analysis. For nutrient determination, water was immediately filtered through a 20 μm filter and concentrations of reactive phosphate, nitrates, ammonia and ammonium were measured using the HACH model DREL 2010 Portable Water Chemistry Apparatus. The phytoplankton samples were preserved with an acetic acid/formalin/alcohol preservative and stored for future analysis and enumeration using the Olympus BX40 System Microscope. Approximately 0.2 ml of the phytoplankton samples (approximately 5-10% of total) were counted to identify the dominant genera. Chlorophyll using methods specified by Wetzel and Likens (1991) and EPA method 445.0 was analyzed. The filters were thawed and then ground using a teflon pestle attached to a motor (500 rpm, 1 min.) in 90% acetone. The slurry was steeped for 24 hours at 4C and then centrifuged at 1000 rpm for 5 minutes. The supernatant was analyzed for direct chlorophyll *a* concentrations corrected for pheopigments. In the early spring experiment the chlorophyll *a* analysis was conducted by Shimdzu DR-3, RF-540 fluorometer at excitation of 440nm and emission of 660nm, and the early and late summer experiments using the Turner Design TD-700 fluorometer for direct concentration by the Welshmeyer non-acidification method at 436nm excitation and 680nm emission. All statistical analysis was performed on SYSTAT 7.0.1.

RESULTS

The physical and chemical parameters between the treated and non-treated enclosures were analyzed with Discriminate Function Analysis (DFA) and Analysis of Variance (ANOVA). The means of the two groups indicated that there were differences in nitrates, ammonia, chlorophyll, depth, specific conductivity, dissolved oxygen percent, and turbidity (Table 1). The standard errors indicated variation between the treated and control enclosures in nitrates (SE 0.106, 0.127), chlorophyll (SE = 9.098, 16.84), specific conductivity (SE = 9.671, 8.569), dissolved oxygen percent (SE = 5.255, 4.754) and turbidity (SE = 1.205, 2.065) (Table 2). In order to verify normal distribution of the means of the control enclosures, t-tests were performed. This analysis is to determine whether the control enclosures before P-addition had the same mean values. The t-statistic for the nutrient chemistry indicated that the control enclosures were not significantly different for the three experiments ($P>0.05$).

Analysis of variance was performed to evaluate differences between the individual variables with respect to P-enrichment. Nitrates were the most significant ($F = 5.972$, $P = 0.001$, $df = 3$), followed by turbidity ($F = 8.329$, $P = 0.005$, $df = 1$). The nutrients ammonium ($F = 4.24$, $P = 0.006$, $df = 3$) and ammonia ($F = 4.144$, $P= 0.007$, $df = 3$) were very significant in comparing the treated and non-treated enclosures. Although chlorophyll was not statistically significant ($F = 3.2$, $p = 0.076$, $df = 1$) there appears to be differences between the treated and non- treated enclosures on some of the specific sampling dates, indicating two individual enclosures responded to P-addition. ANOVAs were also performed on the dominant genera to observe whether P-addition resulted in

significant differences in phytoplankton numbers. In the three experiments there were no significant differences between the treated and non-treated enclosures, $P>0.05$.

The Discriminate Function Analysis indicated that there were overall significant differences between the enriched and control enclosures (Wilks' lambda = 0.74, F = 2.96, df = 12 and p = 0.0014). The classification matrix indicated that a total of 72% of cases in the row categories were classified correctly into columns. The Jackknifed classification matrix indicated that a total of 64 percent of the variables can be placed in correct categories. The classification functions which predict whether a function is in a treated or non-treated category indicated the highest difference between the groups in nitrates and ammonia. The canonical scores were saved and plotted on a histogram to observe the amount of overlap between the two groups. Plotting the score against the frequency (Figure 1) reveals a distinct separation between the control and treated groups, however there is 1-0.72 overlap between the groups (Figure 1).

In the early spring experiment the control enclosures had average N:P ratios of 11:1 by element. Since phytoplankton require N:P elemental ratio of 16:1 for growth (Redfield 1958), and microorganisms require N:P of 10:1, this environment may have been limiting in the amount of nitrogen available for phytoplankton and microorganism growth (Redfield 1958; Tezuka 1990). The treated enclosures had an average N:P ratio of 11.5:1, almost identical to the control, indicating P-addition may not have altered the nitrogen to phosphorus ratios. The early summer experiment on average had a lower N:P ratio compared to early spring. The average N:P for the control enclosures was approximately 4:1, and the average for the treated enclosures was 5:1. During the late summer, the control enclosures had an average N:P of 5:1 and the treated 6:1 (Table 2).

Results suggest that for Chaney Lake, the system is strongly N-limited in respect to algae, however, the same may not be the case for microorganisms; further experimentation is required to answer that question.

The Spearman correlation matrix indicated a high positive correlation between date and chlorophyll in the control group ($r = 0.797$) and date and chlorophyll in the enriched group ($r = 0.847$). In addition, in the control group there were marginal correlations between phosphorus and temperature ($r = 0.688$) and ammonia and ammonium ($r = 0.998$). In the enriched group, there were correlations between phosphorus and temperature ($r = 0.714$), nitrates and ammonia ($r = 0.721$), ammonia and ammonium ($r = 0.998$), and ammonium and dissolved oxygen ($r = 0.952$) (Table 3).

A Pearson Correlation matrix with Bonferroni adjusted probability values was also conducted. This analysis indicated a significant correlation between phosphate and nitrates, ammonia, and ammonium, $P<0.05$. Also, significant correlation between chlorophyll concentrations with temperature and specific conductivity, $P<0.05$.

In the early spring experiment, March 12-April 12, 1998, the phosphorus levels in both the control and treated enclosures increased the week after phosphorus addition, but declined over the remaining course of the experiment (Figure 2). The nitrate concentrations stayed approximately constant in the treated enclosures, but declined in the control enclosures (Figure 3). Following phosphorus addition, ammonia was not affected; however, during the third, fourth, and fifth weeks of the experiment, the control enclosures had a lower concentration than the treated cells (Figure 4). Altogether, the ammonia concentrations decreased in both control and treated enclosures. In the early spring experiment ammonium followed the same pattern as ammonia. Chlorophyll

concentrations for both treated and non-treated enclosures remained relatively constant through most of the early spring experiment, except for the very last sampling date when they were the highest-probably indicating the beginning of optimum temperatures for algal growth (Figure 5). During the last week of sampling there were higher concentrations of chlorophyll in the control enclosures than in treated enclosures (Figure 5). Depth did not show a pattern of variation during the course of this experiment. Temperature increased steadily during the course of the experiment except between the March 26th and April 5th sampling dates when the water temperature was lower than in previous weeks (Figure 6). There were no significant differences in the values for specific conductivity through the course of the experiment. Dissolved oxygen and dissolved oxygen percent decreased following phosphorus enrichment, increased in the fourth week and slightly decreased the last week of sampling (Figure 7). There were no large differences between the control and treated enclosures for DO and DO%. The levels of pH stayed constant throughout the experiment, 6.3 to 6.4. Turbidity in this experiment showed significant differences between the control and treated enclosures following phosphorus enrichment, especially in the last three weeks of the experiment (Figure 8). The P-treated enclosures were more turbid.

In this experiment, diatoms (Bacillariophyceae) and cyanophytes (blue-green algae) were the most common groups present in thephytoplankton. *Anacystis, Microcystis,* and *Anabaena* species were the most common blue-green algae. *Anabaena* numbers increased slightly in the treated enclosures in the fourth week of the experiment (Figure 9). As regards to diatoms, only *Eunotia* species on the sampling date April 5, 1998 had higher numbers in enclosures with phosphorus added to them (Figure 10). Among

chlorophytes (green algae), *Planktosphaeria* and *Closterium* were the only organisms present, and neither seemed affected by P treatment (Figure 11). Shannon-Weaver diversity indices (H) were calculated to observe whether the two treatments differed in the amount of phytoplankton diversity. In the early spring experiment, diversity as measured by these metrics indicated slight differences between the control and P-enriched groups of phytoplankton (Table 4). Overall, it seems phosphorus enrichment only slightly increased phytoplankton composition or diversity in early spring ($P>0.05$).

In the early summer experiment (May 21–June 23, 1998), phosphorus levels fluctuated following enrichment, followed by an increase in the third and fifth weeks and with a decrease in the fourth week (Figure 12). There were no significant differences in the concentrations of phosphorus in the treated and non-treated enclosures. The concentration levels for nitrates also fluctuated following phosphorus enrichment; however, they steadily increased during the course of the experiment. There were also statistically significant differences in concentrations of nitrates between the control and treated enclosures (Figure 13). Ammonia and ammonium followed the same pattern as the nitrates. The chlorophyll *a* concentrations increased during the course of this experiment. The major differences between the control and treated enclosures were in the fourth and fifth weeks of the experiment (Figure 14). This increase in standard error can reduce the chance for significance during individual dates between treated and not-treated enclosures. There were no differences in depth, pH, and specific conductivity throughout this experiment. The water temperature ranged from 21.5C to 24.5C throughout the duration of the experiment (Figure 15). Dissolved oxygen ranged from 3.5 to 14 mg/l with no significant differences between treatments, although there was

variation throughout the experiment (Figure 16). Although there were no major changes in turbidity through the course of the experiment, there were significant differences in turbidity (Figure 17) between the treated and non-treated enclosures ($P<0.05$).

Anabaena species dominated the phytoplankton, and they also responded slightly to phosphorus enrichment immediately after P was added. *Oscillatoria* numbers also seem to increase after enrichment; however, neither of these genera of blue-green algae were significantly different with respect to P treatment ($P>0.05$) (Figure 18). Filamentous green algae also were present in considerable numbers in this experiment, specifically two species of *Mougeotia*, one species of *Zygnema*, two species of *Oedogonium*, and one species of *Hyalotheca*. There were also two species of the chrysophyte *Dinobryon*, and the chlorophyte *Planktosphaeria*. *Mougeotia* species and *Dinobryon* species, although abundant, did not show an increase in numbers in respect to enrichment by phosphorus (Figure 19). Diatoms, specifically *Eunotia* and *Pinnularia*, increased just slightly with phosphorus enrichment (Figure 20), but the changes were not statistically significant ($P>0.05$). Neither the Shannon-Weaver diversity index nor the equitability index indicated high levels of difference between control and treated enclosures (Table 4).

The late summer experiment was conducted between July 31 and August 20, 1998. Following the last sampling date, the water in the open marsh area of Chaney Lake receded completely. There was no change in the phosphorus levels following treatment (Figure 21). In the third week, the phosphorus levels were much lower than the previous weeks, most likely because the depth of the marsh at this point was very low. Nitrogen concentrations in the treated enclosures decreased, while the control enclosures increased and then decreased following treatment (Figure 22). Ammonia and ammonium

concentrations showed the same pattern as the nitrates. Chlorophyll *a* stayed constant the week following phosphorus enrichment, although the treated enclosures were decreasing the last week (Figure 23). Depth, temperature, specific conductivity, and pH did not show differences between the treated and control enclosures. Dissolved oxygen and dissolved oxygen % did not show significant differences between the control and treated enclosures. Turbidity did indicate significant differences between the treated and non-treated enclosures. The treated enclosures were more turbid than the control (Figure 24).

In this experiment *Anacystis* and *Oscillatoria* were the two blue-green algae that responded slightly to phosphorus enrichment (Figure 25). Also present in both the control and treated enclosures in abundance were species of *Oedogonium, Mougeotia, Scenedesmus*, and *Closterium.* However, there were no significant differences between the two treatments (Figure 26). The diatoms, *Eunotia* and *Pinnularia*, responded slightly to phosphorus enrichment (Figure 27). Again the differences were not statistically significant ($P>0.05$). The Shannon-Weaver diversity index also indicated the same level of diversity between the two treatments. However, in comparing the three experiments, the early spring and summer experiments which were conducted for the same amount of time had similar diversity (H) and evenness values (J). The late summer indices were higher, indicating more diversity later in the season in respect to phytoplankton genera present (Table 4).

DISCUSSION

In the past, no study has been conducted on phosphorus enrichment and its effect on the phytoplankton communities of Chaney Lake. In this study it was determined that the phosphorus levels in the marsh area of Chaney Lake may not be limiting to phytoplankton. The enclosures enriched with phosphorus did not show significant differences in phytoplankton community structure compared to the non-treated enclosures. Furthermore, chlorophyll *a* concentrations did not reflect that phosphorus addition affected algal biomass. Soluble reactive phosphate levels themselves were not significantly different between the control and P-added mesocosms. Since phosphorus may be in adequate supply in this environment, enrichment may not produce a shift in phytoplankton community structure. Overall, in analyzing the organisms in the control enclosures (reflecting the marsh's natural environment) there was low algal diversity (Gary Dillard, personal communication). In fact, this system was mostly dominated by two genera of blue green algae, *Anabaena* and *Oscillatoria*, the green algae *Mougeotia*, *Zygnema*, and *Oedogonium*, and the diatoms *Synedra*, *Eunotia* and *Pinnularia*. Algal composition and community structure were not altered in response to p-addition.

While phosphorus may not be limiting to phytoplankton, it may be limiting to microbes, periphyton, or both. The PO_4 concentrations and the N:P ratios of the three separate experiments suggest that in the marsh area of Chaney Lake phosphorus may not be the most limiting nutrient for phytoplankton growth. Even though nitrogen enrichment was not tested, by observing the nitrates/ammonia concentrations and N:P ratios, conclusion can be drawn that nitrogen may be the limiting nutrient in this system for algae. Whereas we did not directly observe the bacterioplankton in Chaney Lake, the

N:P ratios leads one to speculate that the microbial communities are N-limited. The N-limitation would begin to explain the significant differences in nitrates and ammonia concentrations and turbidity values of the treated and control enclosures. Bacteria play very important roles in the nitrogen cycle. In the process of nitrification, nitrifying bacteria are biological catalysts, oxidizing ammonia to nitrate. Nitrification is a major aerobic process and occurs readily in highly oxygenated environments such as well-drained soils with neutral pH. If materials high in protein, such as manure or sewage, are added to the environment the rate of nitrification is increased. Although nitrate is readily assimilated in plants, it is very water-soluble and is rapidly leached from soils receiving high rainfall (Brock 1997). Ammonia is produced during the decomposition of organic nitrogen compounds (denitrification/ammonification) and exists at neutral pH as the ammonium ion. Under anoxic conditions ammonia is stable, and it is in this condition that nitrogen predominates in anoxic environments (Brock 1997). In aerobic conditions, ammonia can be oxidized to nitrogen oxides and nitrate with catalysts and strong oxidizing agents (Brock 1997). Because the immediate area enclosing our study site is surrounded with agricultural fields, and Chaney Lake is located at the headwater area of land used mainly for farms and pastures, it would be reasonable to predict that the bacterioplankton community is the most active one in this system.

In a study of a eutrophic Norwegian Lake, Vadstein et al. (1988) found that bacteria had higher consumption and requirements of P than phytoplankton, and bacteria acted as consumers of inorganic P. Their net consumption of P was four times higher than that of the phytoplankton (Vadstein et al 1988). Bacteria are generally looked upon as "mineralizing" organisms, making organic nutrients available for the primary producers

through oxidation and the subsequent release of the inorganic form. The bacterial community in this Norwegian Lake was P limited for most of the investigated period. The high P requirements of bacteria and the relatively low P:C ratio of both dissolved and particulate substrate available for bacterial growth indicate that the bacteria were consumers of phosphate rather than functioning as "remineralizing" organisms (Vadstein et al. 1988). In a study of literature and field data, Currie (1990) observed large-scale variation and interactions among phytoplankton, bacterioplankton, and phosphorus. Currie postulated an alternative describing the relationship between phytoplankton and bacterioplankton. Past studies have indicated that total P abundance determines algal abundance, which in turn determines bacterial abundance, or that algae or bacteria compete for P. Currie found that the data were most consistent with an alternative model, postulating that P directly influences both algae and bacterial abundance, that algae and bacteria directly influence each other's abundance, and that a third factor (temperature or bacteriovore abundance) also influences algae and bacteria in the same manner (Currie 1990).

Toolan et al. (1991) conducted a study to observe whether bacterioplankton production was increased because of inorganic phosphorus addition in a small meso-eutrophic lake located in southern New York. Traditionally, availability of organic carbon has been thought of as the key limiting factor in bacterial growth. However, the high phosphorus requirement of bacteria relative to that of phytoplankton and the percentage of uptake, 72 to 98% (Vadstein 1988, Currie 1984) traceable to bacteria suggest that the dissolved inorganic phosphorus (DIP) supply could also limit bacterial production (Toolan et al. 1991). Toolan et al. (1991) found that addition of DIP

significantly stimulated bacterial production, with strong DIP responses in all of their experiments. Also, addition of KH_2PO_4 caused three-eightfold increases in the rate of incorporation of [^{3}H]TdR into DNA. They also found that addition of as little as 0.05 μmol of P was sufficient to meet the P demands of bacteria (Toolan et al. 1991).

In a study by Morris and Lewis (1992), the researchers investigated nutrient limitation of bacterioplankton growth in Lake Dillon, Colorado. They found that bacterioplankton population growth rates were highly correlated with P concentrations but not as much as with dissolved organic C concentrations. Bacterioplankton growth in the summer epilimnion responded strongly to the addition of P alone or in combination with N or labile organic C. The experimental and statistical analyses indicated that P, rather than organic C, is the critical nutrient for bacterioplankton growth in Lake Dillon, Colorado (Morris and Lewis 1992).

Another study observing the phytoplankton and bacterioplankton relationship with respect to nutrient enrichment was by Le et al. (1994). In this study, experiments were conducted with large mesocosms containing whole plankton communities from mesoeutrophic Calder Lake. A range of N and P concentrations and N/P ratios were tested to examine coupling between the biomass of planktonic bacteria and that of phytoplankton under nutrient-limited and nutrient-surplus conditions. It was hypothesized that the growth of both bacteria and phytoplankton can be stimulated directly and separately by N or P loading, and increases in bacterial growth can occur independently of those of phytoplankton. The correlation between bacterial abundance and phytoplankton (as chlorophyll *a*) as affected by the different abilities of these organisms to obtain inorganic P was also investigated. It was shown that bacterial

abundance in fresh waters could be stimulated by inorganic nutrients. They found that bacterial production could be stimulated by P addition alone without being coupled to increases in phytoplankton and without N addition (Le et al. 1994). Further proof can be seen in a nonsignificant regression between bacterial numbers and phytoplankton chlorophyll *a* level in systems without added N. In this experiment, addition of P without N stimulated the growth of planktonic bacteria but did not stimulate the growth of phytoplankton. Three consecutive weeks of apparent P limitation in bacteria, while phytoplankton groups experienced only one or two such periods, suggested that bacteria are not superior competitors for low P supplies in mixed communities (Le et al. 1994). Bacterioplankton may simply be unable to store polyphosphates as efficiently as most algal species, making them dependent on a more continuous supply of dissolved inorganic phosphates. Le et al. (1994) observed that bacteria respond positively and significantly to P addition, apparently regardless of external dissolved inorganic N pools. When N is added, both bacteria and phytoplankton can respond to P addition, and so their growth appears coupled. The suggestion is that N and P conditions are mutual factors affecting algal-bacterial coupling, which elicit independent but parallel increases in bacterial and phytoplanktonic levels (Le et al. 1994). In this study the conclusion was that coupling bacterial and phytoplankton production could depend on both N and P status. In systems with low N/P ratios, bacteria are more responsive to pulses of inorganic P, and hence the two components become uncoupled. In systems with higher N/P ratios, both components of the community should respond to P dynamics. The necessity of greater N in this dynamic suggests that a high N/P ratio is necessary for a close coupling between bacteria and phytoplankton in freshwater (Le et al. 1994).

Elser et al. (1995) studied elemental ratios and the uptake and release of nutrients by phytoplankton and bacteria in three lakes of the Canadian shield. The dynamics of carbon, nitrogen, and phosphorus elemental ratios, and dark uptake/release of N and P in bacterial and phytoplankton size fractions during the summer of 1992 were observed. These lakes were of contrasting food web structure and trophic state (Elser et al. 1995). When the N-flux data in the bacterial size fraction relative to the N/P ratio of the bacteria were observed, it was revealed a threshold N/P ratio (~22:1 N/P, by atoms) was found, below which bacteria took up and sequestered added N, and above which N was released (Elser et al. 1995). Elser et al. (1995) based their studies on earlier work by Tezuka (1989, 1990), who indicated that the functional role of aquatic bacteria in the nitrogen (N) and phosphorus (P) cycles depends on the elemental quality of organic nutrient substrates available. When substrates have a high P-content, bacteria mineralize P and retain N. On the other hand, when organic substrates are low in P, bacteria retain P and disproportionately release N. Thus, the functional role of bacteria in the N and P cycles, as source or sink, may depend on the stoichiometric characteristics of the substrate compared to the demand of the cell's physiology (Tezuka 1989, 1990).

The analysis of data set forth by these studies finds a consistent relationship between bacterioplankton abundance and phosphorus levels. Specifically, the studies conducted by Le et al. (1994) and Elser et al. (1995) observe the importance of N:P stoichiometric ratios for bacterioplankton. The focus of the studies mentioned involve lake environments that are different from Chaney Lake's open marsh. However, substantial contributions and the role of bacterioplankton to the availability and uptake of nutrients can be related to Chaney Lake. In respect to the low N:P ratios and the significant

differences in nitrates and turbidity and the coupling aspects of N and P mentioned in the study conducted by Le et al. (1994) we can assume the important role bacterioplankton have in nutrient utilization and uptake in the marsh area of Chaney Lake.

The study in Chaney Lake was a preliminary research observing P-enrichment and its effects on algae. The next step in studying this temporary wetland would be to investigate the role of planktonic bacteria and periphyton. Further research is needed in identifying the bacterioplankton and periphyton communities and whether there is a relationship between availability of nutrients and these groups of organisms. Chaney Lake's unique hydrogeology offers an opportunity to study an environment with both aquatic and terrestrial characteristics that is very different from either of those individual habitats. Quantification of the role of bacterioplankton and periphyton in Chaney Lake and how they relate to nutrient availability need to be further investigated. Present results suggest the importance bacterioplankton may play in the nutrient availability in Chaney Lake.

APPENDIX

Group Means	***Control***	***Treated***
Phosphate (mg/l)	0.767	0.746
Nitrates (mg/l)	2.539	3.029
Ammonia (mg/l)	0.647	0.761
Ammonium (mg/l)	0.685	0.805
Chlorophyll (μg/l)	40.77	76.45
Depth (cm)	48.78	46.99
Temperature (°C)	19.11	18.52
SPC (μohms)	393.13	397.93
Dissolved Oxygen %	67.41	70.43
Dissolved Oxygen (mg/l)	6.33	6.714
PH	6.34	6.325
Turbidity (ntu)	14.39	21.55

Group Standard Errors	***Control***	***Treated***
Phosphate (mg/l)	0.058	0.056
Nitrates (mg/l)	0.106	0.127
Ammonia (mg/l)	0.029	0.032
Ammonium (mg/l)	0.030	0.034
Chlorophyll (μg/l)	9.098	16.840
Depth (cm)	1.599	1.832
Temperature (°C)	0.841	0.825
SPC (μohms)	9.671	8.569
Dissolved Oxygen %	5.225	4.754
Dissolved Oxygen (mg/l)	0.493	0.488
PH	0.031	0.021
Turbidity (ntu)	1.205	2.065

Table 1: Means and standard errors for nutrient chemistry, physical parameters, and chlorophyll.
The values include all three experiments-March 12-August 20, 1998.

Date	***Control***	***Treated***
3/12/98	11:1	11:1
3/18/98	6:1	8:1
3/26/98	8:1	12:1
4/5/98	16:1	13:1
4/12/98	14:1	13:1
5/21/98	3:1	4:1
5/29/98	4:1	6:1
6/5/98	4:1	4:1
6/15/98	4:1	6:1
6/23/98	4:1	5:1
7/31/98	4:1	6:1
8/4/98	5:1	5:1
8/20/98	6:1	6.5:1

Table 2: Nitrogen-phosphorus elemental ratios for control and P-treated enclosures, weekly values. Chaney Lake, March 12-August 20, 1998.

Control

	Ammonia	Ammonium	Chl	Date	DO	Nitr	Phos	Temp
Ammonia	1.00							
Ammonium	0.998	1.00						
Chl	0.088	0.097	1.00					
Date	0.052	0.062	0.797	1.00				
DO	-0.135	-0.140	0.022	-0.06	1.00			
Nitr	0.448	0.450	0.043	0.039	-0.03	1.00		
Phos	0.300	0.293	0.419	0.388	-0.23	0.503	1.00	
Temp	0.159	0.162	0.549	0.730	-0.26	0.232	0.688	1.0

Enriched

	Ammonia	Ammonium	Chl	Date	DO	Nitr	Phos	Temp
Ammonia	1.00							
Ammonium	0.998	1.00						
Chl	0.193	0.187	1.00					
Date	0.041	0.032	0.847	1.00				
DO	0.093	0.102	-0.225	-0.10	1.00			
Nitr	0.721	0.718	0.168	-0.01	0.077	1.00		
Phos	0.400	0.393	0.457	0.406	-0.22	0.565	1.00	
Temp	0.221	0.210	0.748	0.745	-0.37	0.330	0.714	1.0

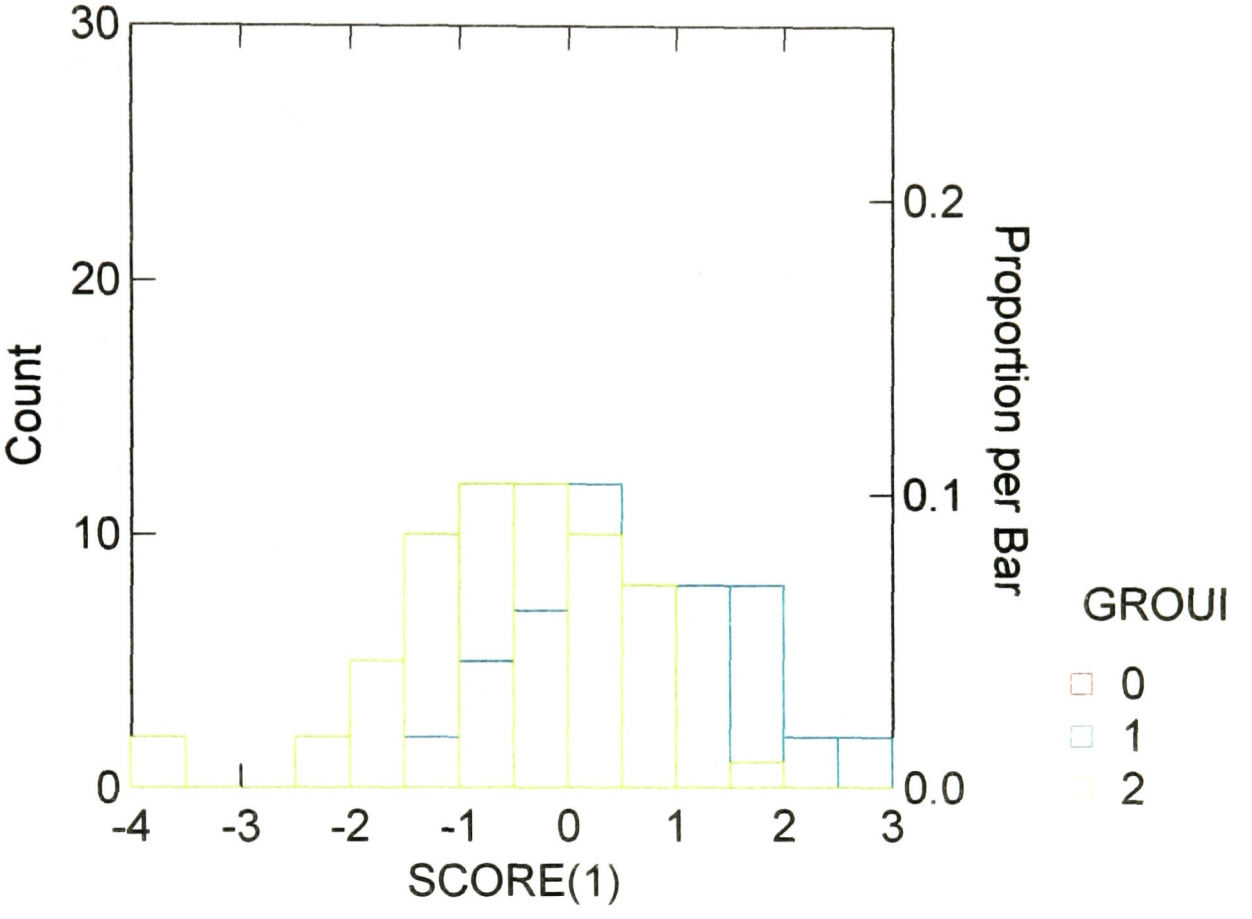
Table 3: Spearman Correlation Matrix. Control and P-enriched enclosures. March 12-August 20, 1998.

Control	*Treated*
Early Spring H =1.81 J = 0.67	 H = 1.94 J = 0.72
Early Sum. H = 1.80 J = 0.668	 H = 1.87 J = 0.69
Late Summer H = 2.51 J = 0.839	 H = 2.41 J = 0.805

Table 4: Shannon-Weaver Diversity index, H-diversity in terms of the number of phytoplankton genera and the spread of individuals.
Equitability index, J-measure of population evenness.

FIGURE 1: Canonical Scores and frequency. This figure shows there is separation between the groups; however with 0-0.72 overlap.

FIGURE 2: Mean phosphate concentrations (mg/l)- Early spring experiment, March 12-April 12, 1998.


30
20
10
0
Count
0.2
0.1
0.0
Proportion per Bar
-4
-3
-2
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0
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SCORE(1)
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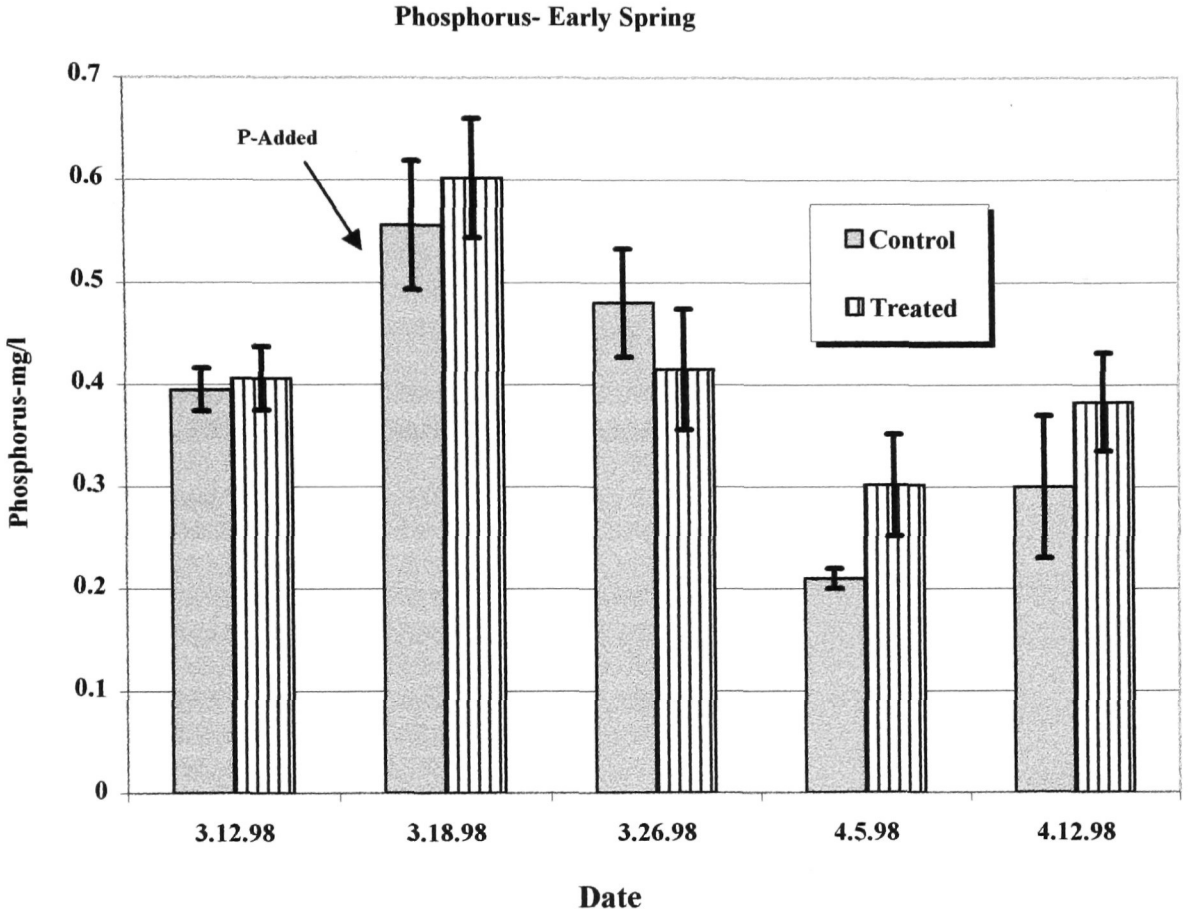

FIGURE 2: Mean phosphate concentrations (mg/l)- Early spring experiment, March 12 - April 12, 1998.

FIGURE 3: Mean nitrates (NOx) concentrations (mg/l)- Early spring experiment, March 12-April 12, 1998.

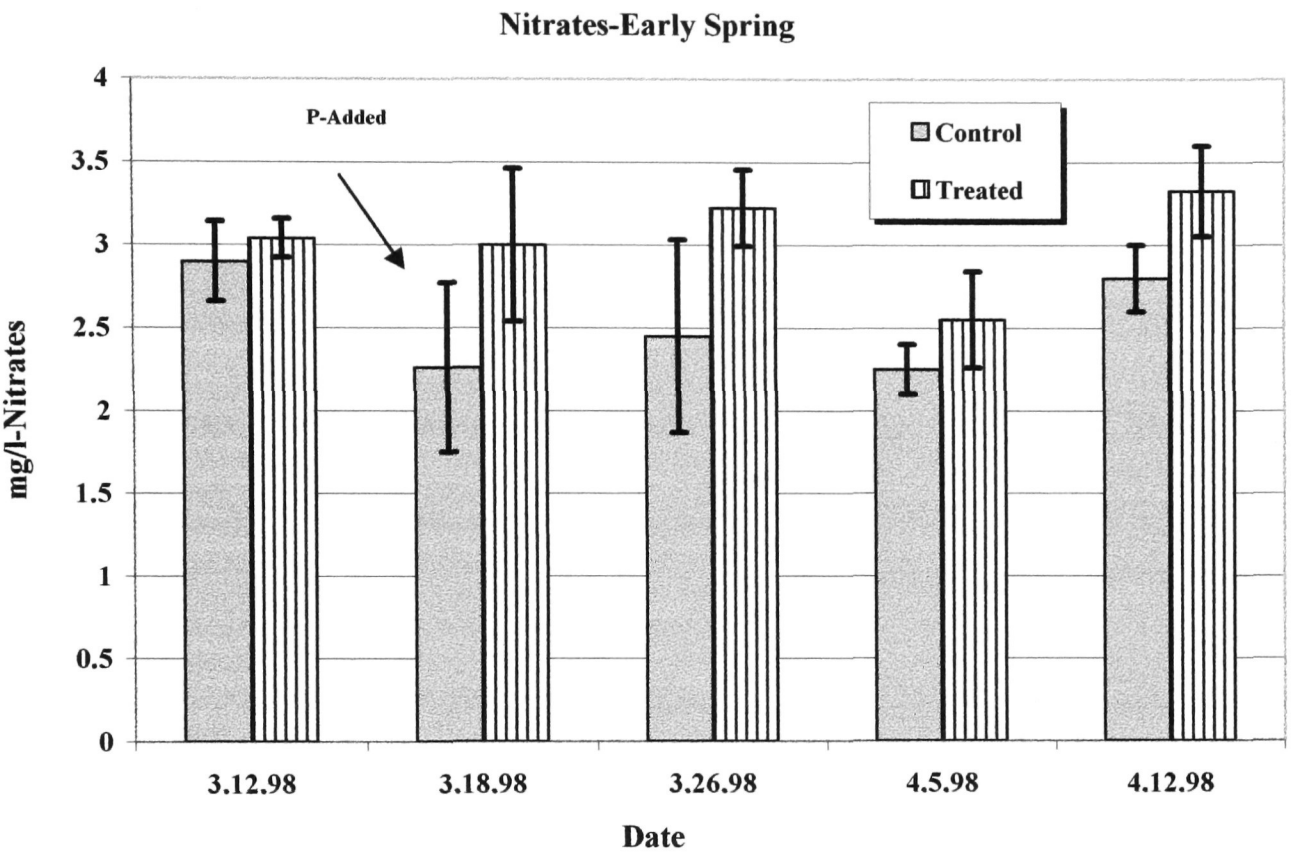

FIGURE 3: Mean nitrates (NOx) concentrations (mg/l) -Early spring experiment, March 12-April 12, 1998.

FIGURE 4: Mean ammonia concentrations (mg/l)- Early spring experiment, March 12-April 12, 1998.

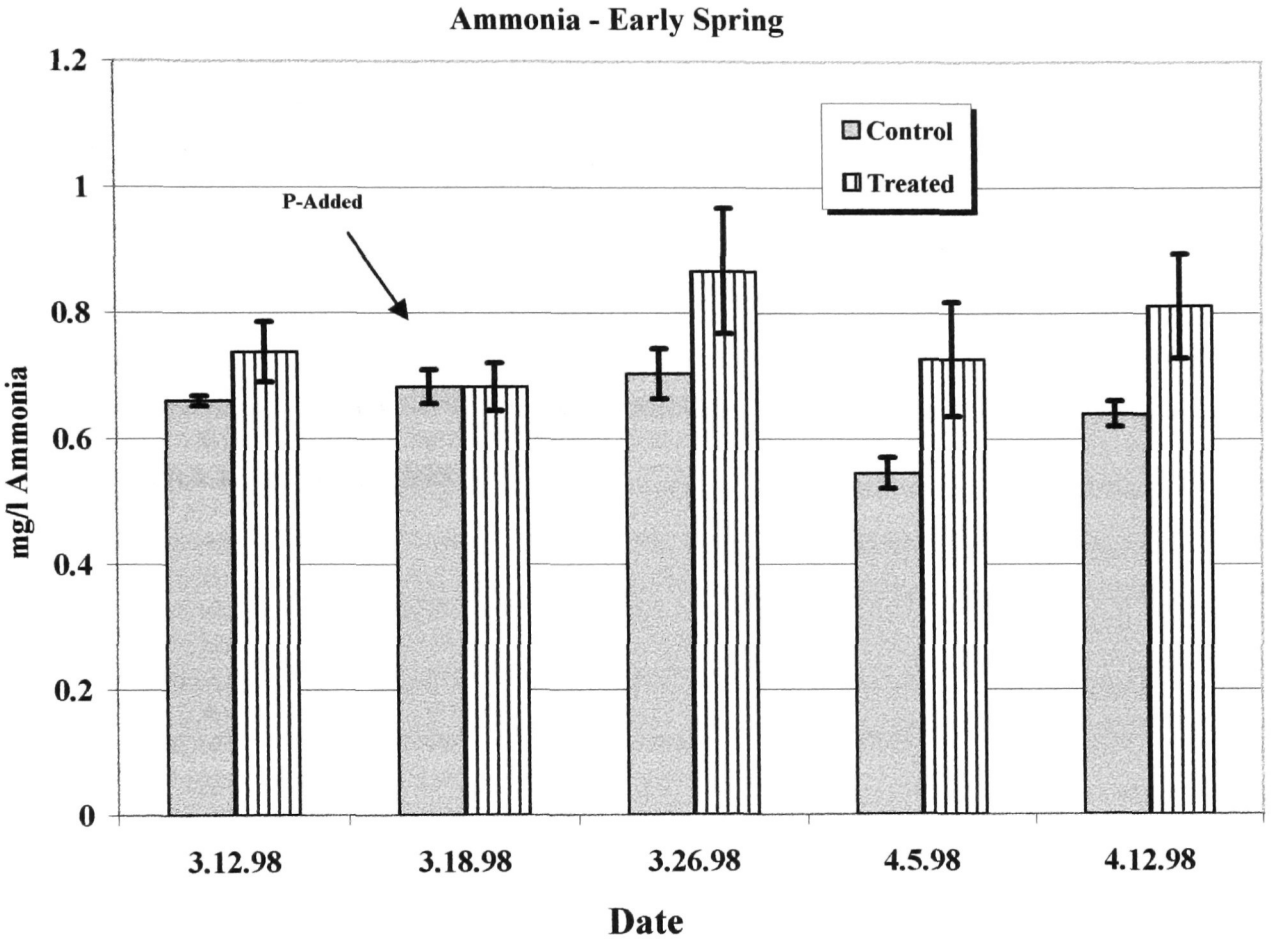

FIGURE 4: Mean ammonia concentrations (mg/l) - Early spring experiment,
March 12 - April 12, 1998.

FIGURE 5: Mean chlorophyll *a* concentrations (micrograms/l)-Early spring experiment, March 12- April 12, 1998.

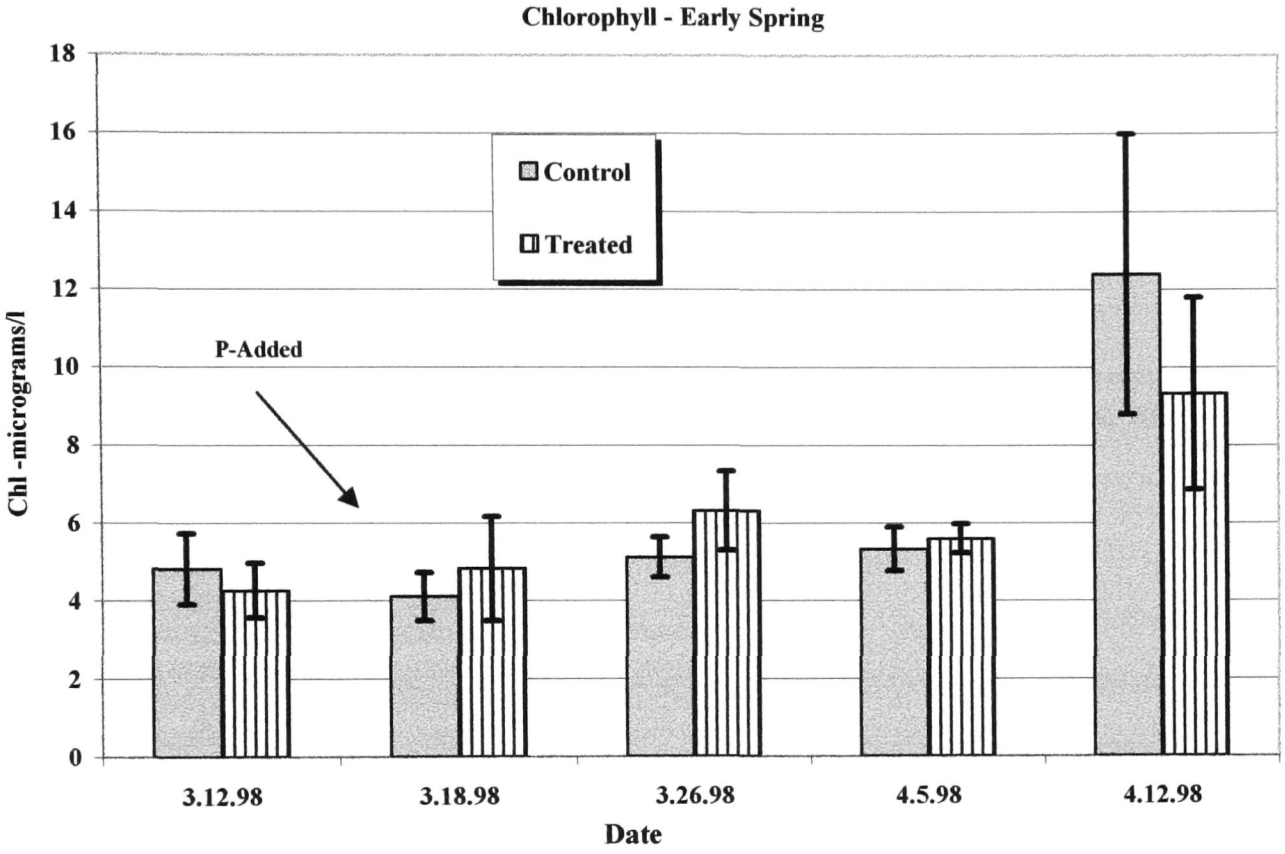

FIGURE 5: Mean chlorophyll *a* concentrations (micrograms/l)-
Early spring experiment, March 12 - April 1998.

FIGURE 6: Mean temperature (degrees Celsius)-Early spring experiment, March 12-April 12, 1998.

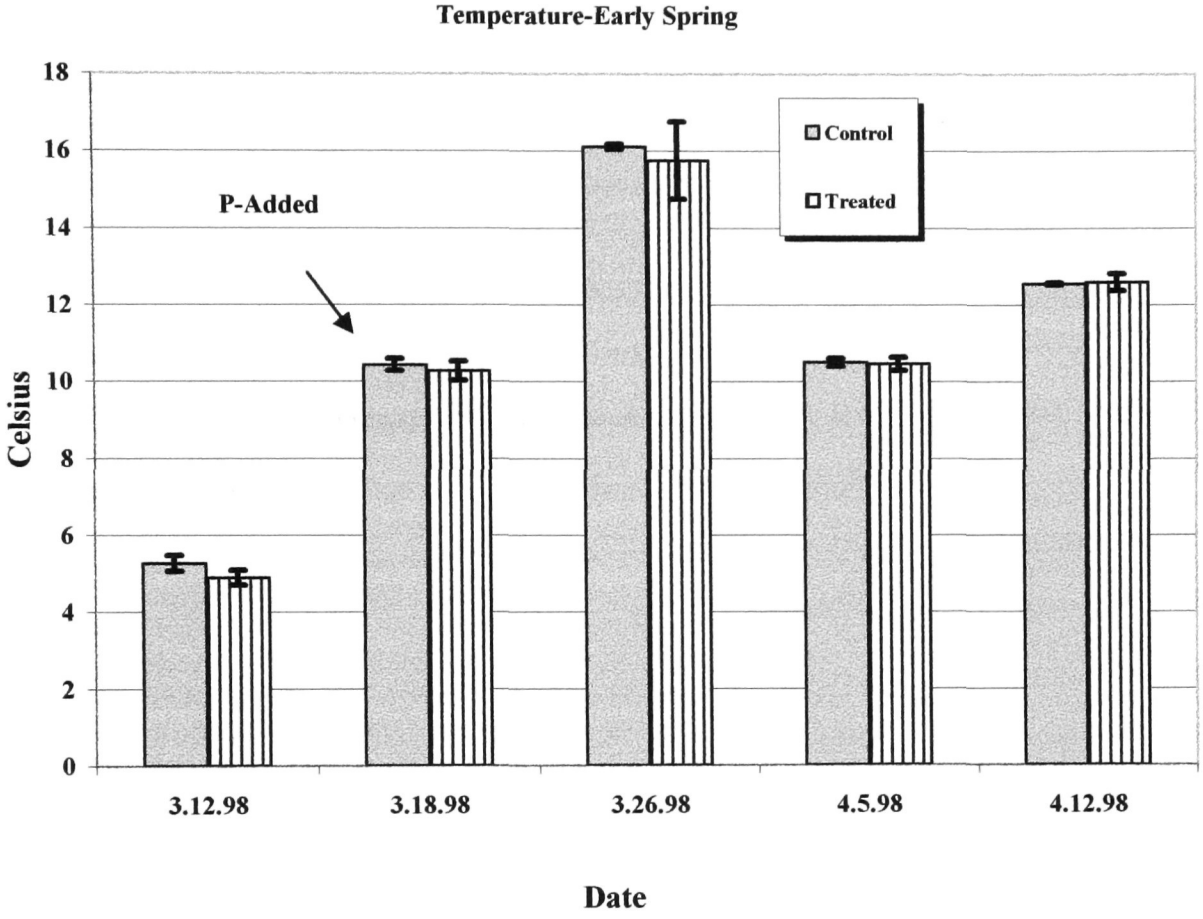

FIGURE 6: Mean Temperature (degrees Celsius)-Early spring experiment, March 12 - April 12, 1998.

FIGURE 7: Mean concentration dissolved oxygen-Early spring experiment, March 12-April 12, 1998.

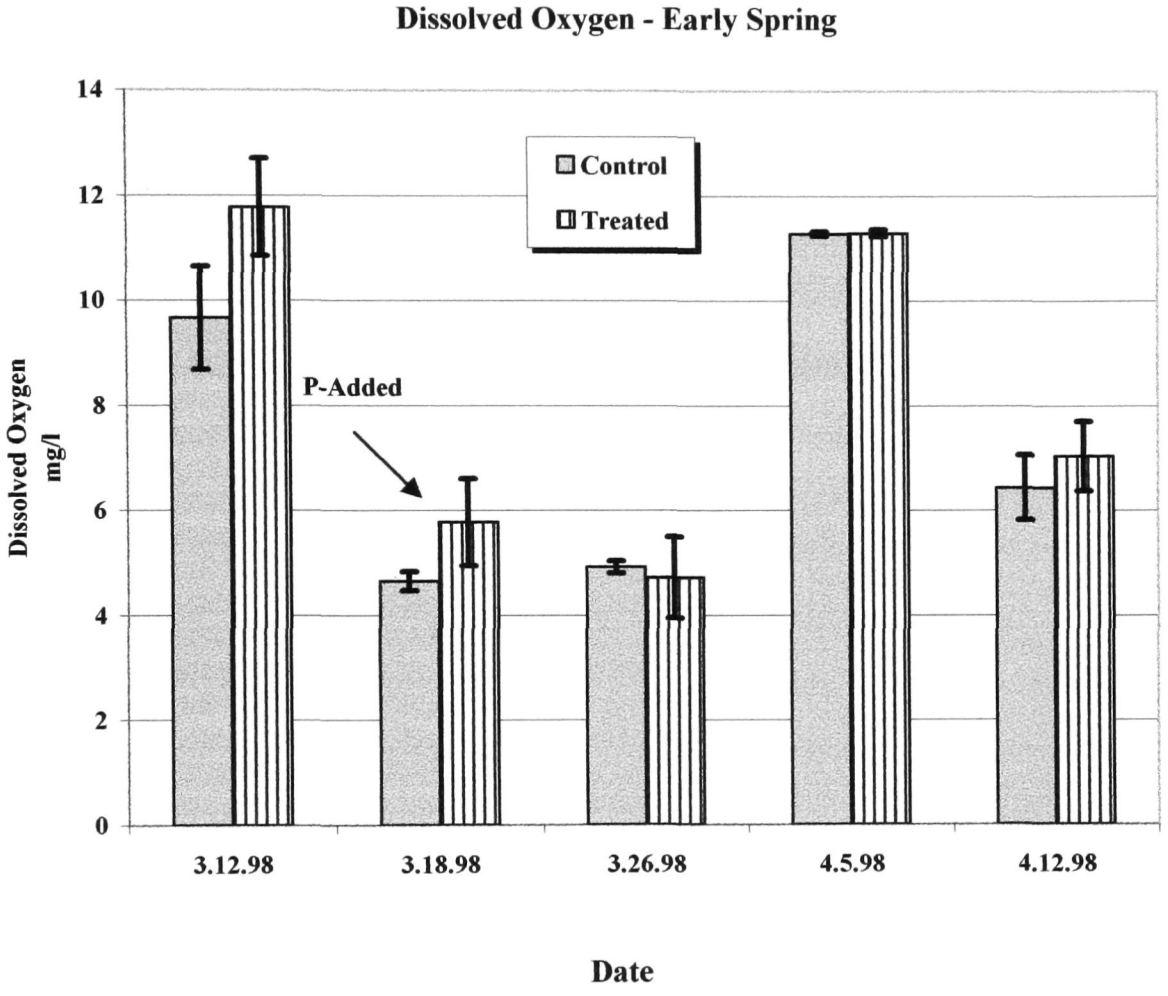

FIGURE 7: Mean concentration dissolved oxygen-Early spring experiment, March 12-April 12, 1998.

FIGURE 8: Mean turbidity (NTU)- Early spring experiment, March 12-April 12, 1998.

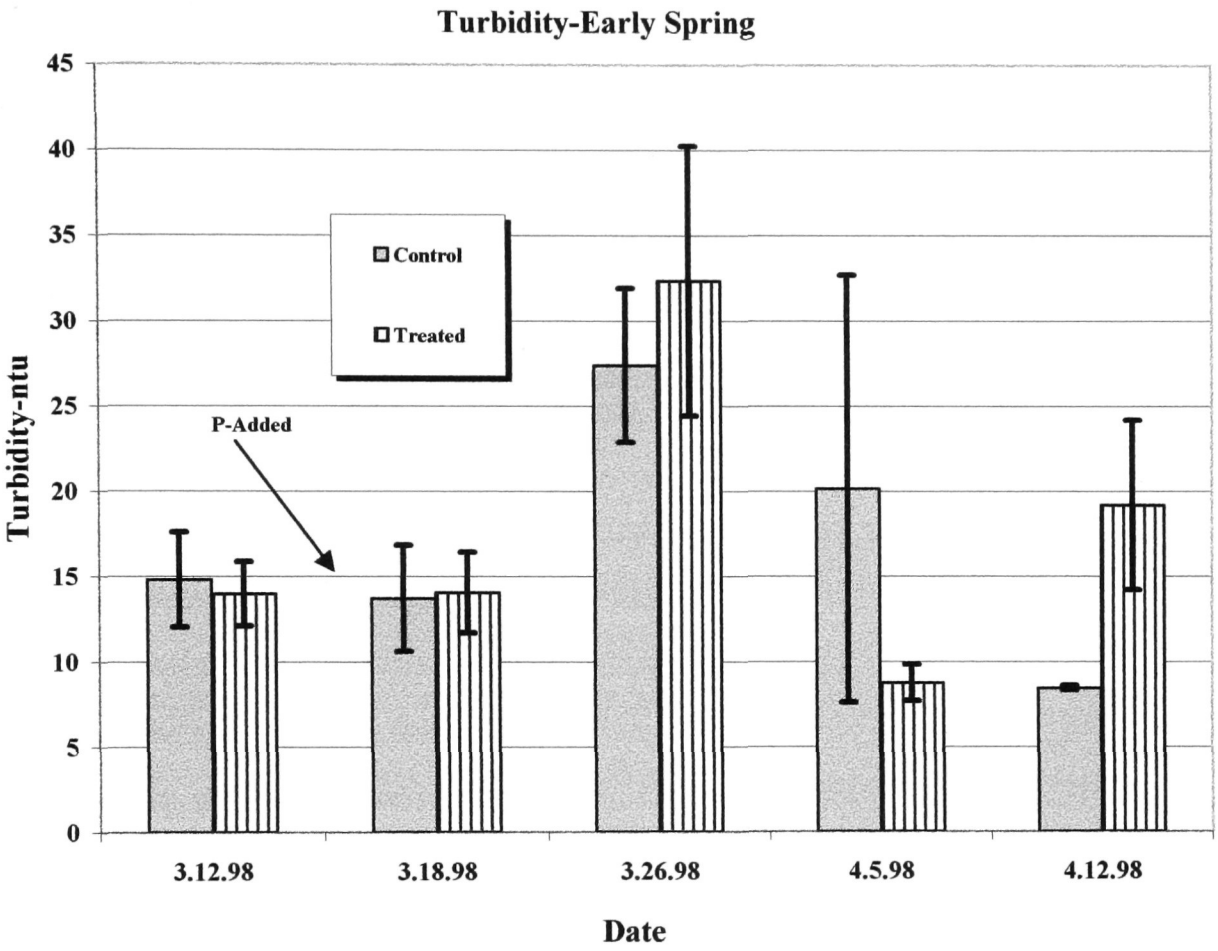

FIGURE 8: Mean turbidity (NTU)-Early spring experiment, March 12 - April 12, 1998.

FIGURE 9: Cyanophyceae-blue-green algae: (Mean # of organism)-Early spring, March 12-April 12, 1998.

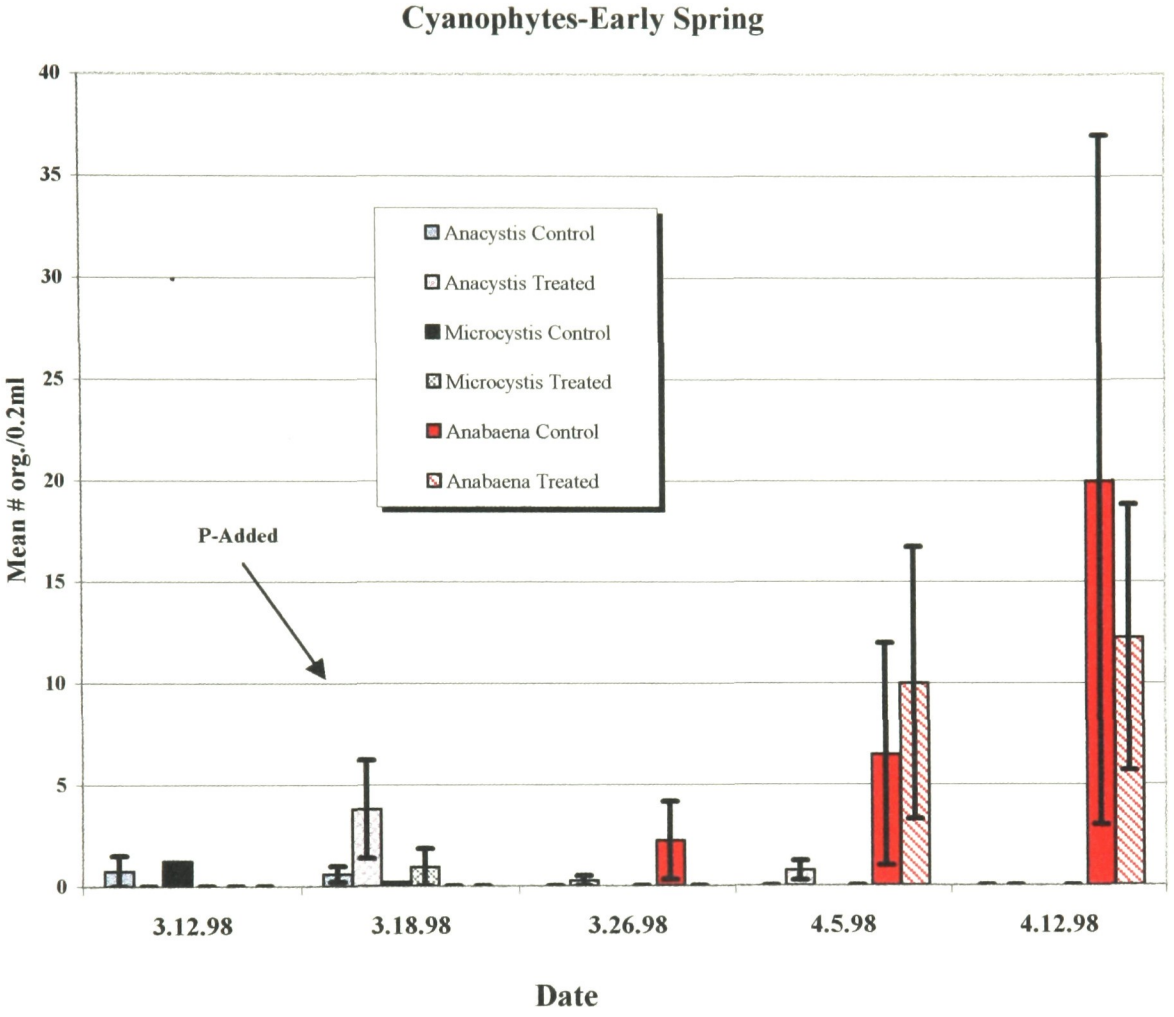

FIGURE 9: Cyanophyceae-blue-green algae: Mean # organisms-Early spring-March 12 -April 12, 1998.

FIGURE 10: Bacillariophyceae-Diatoms. Mean # organisms/0.2ml. Early Spring-March 12-April 12, 1998.

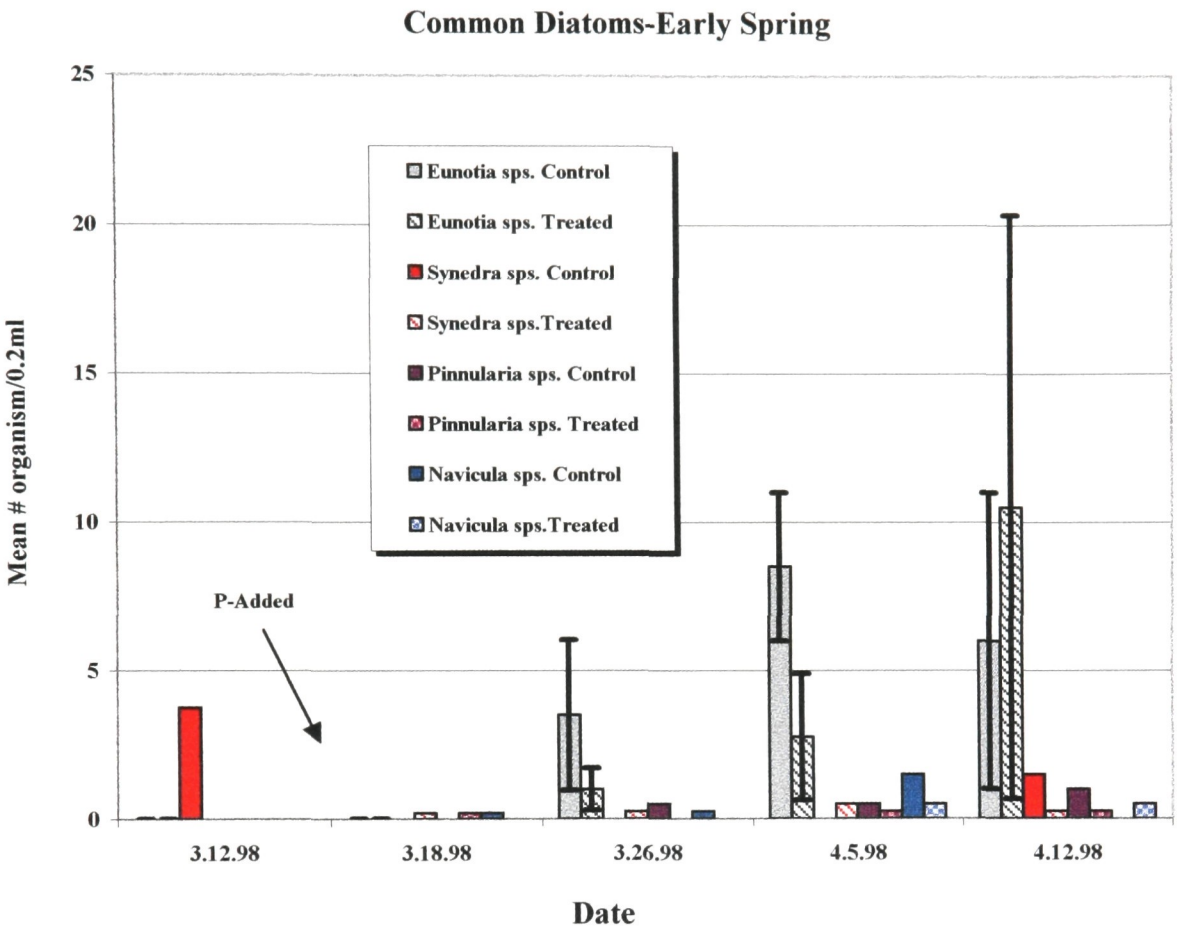

FIGURE 10: Bacillariophyceae-Diatoms. Mean # organism/0.2ml. Early spring-March 12-April 12, 1998.

FIGURE 11: Chlorophyceae-green algae. Mean # organisms/0.2ml. Early spring-March 12-April 12, 1998.

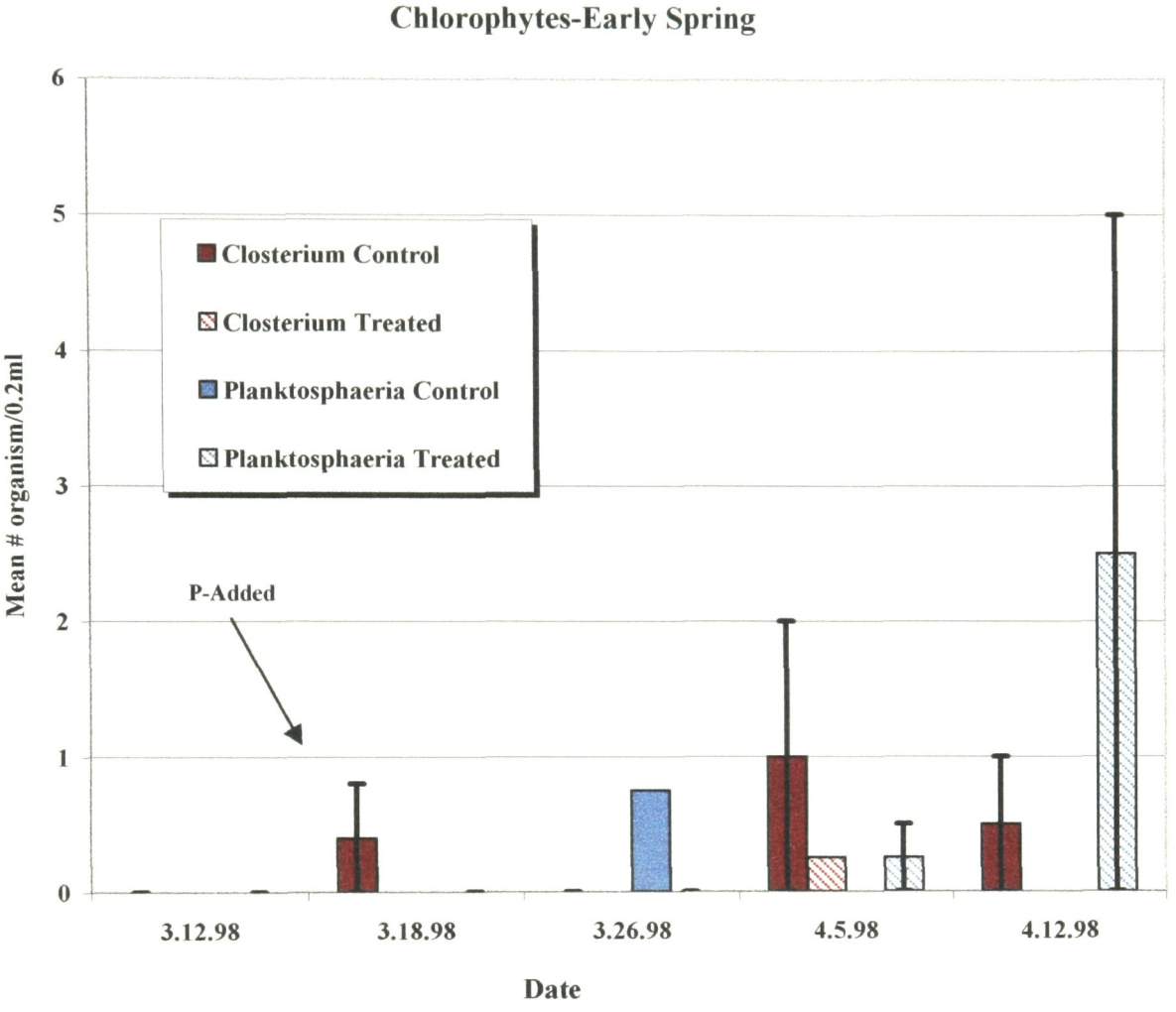

FIGURE 11: Chlorophyceae-green algae. Mean # organisms/0.2ml. Early spring-March 12-April 12, 1998.

FIGURE 12: Mean phosphate concentrations (mg/l)-Early summer experiment, May 21-June 23, 1998.

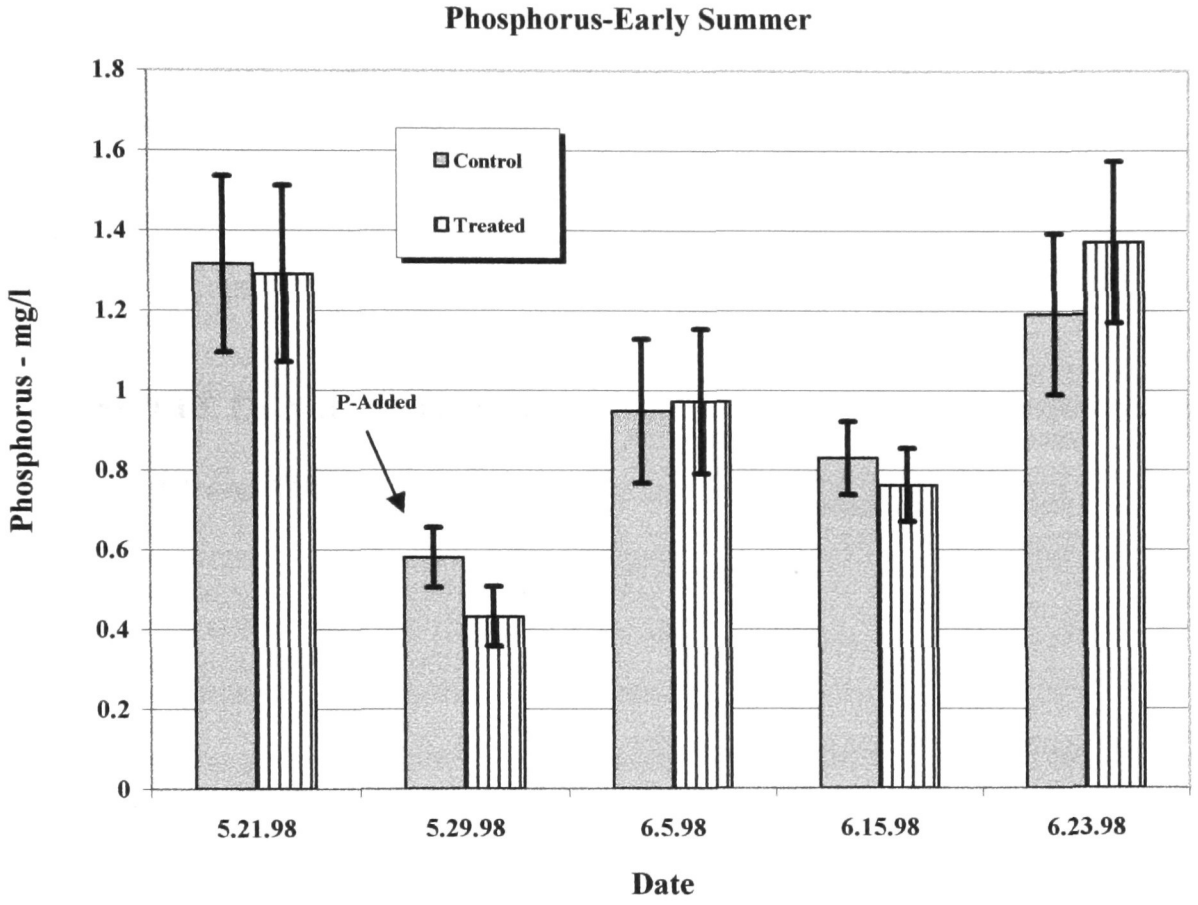

FIGURE 12: Mean phosphate concentrations (mg/l)-Early summer experiment, May 21-June 23, 1998.

FIGURE 13: Mean nitrate (NOx) concentrations (mg/l)-Early summer experiment, May 21-June 23, 1998.

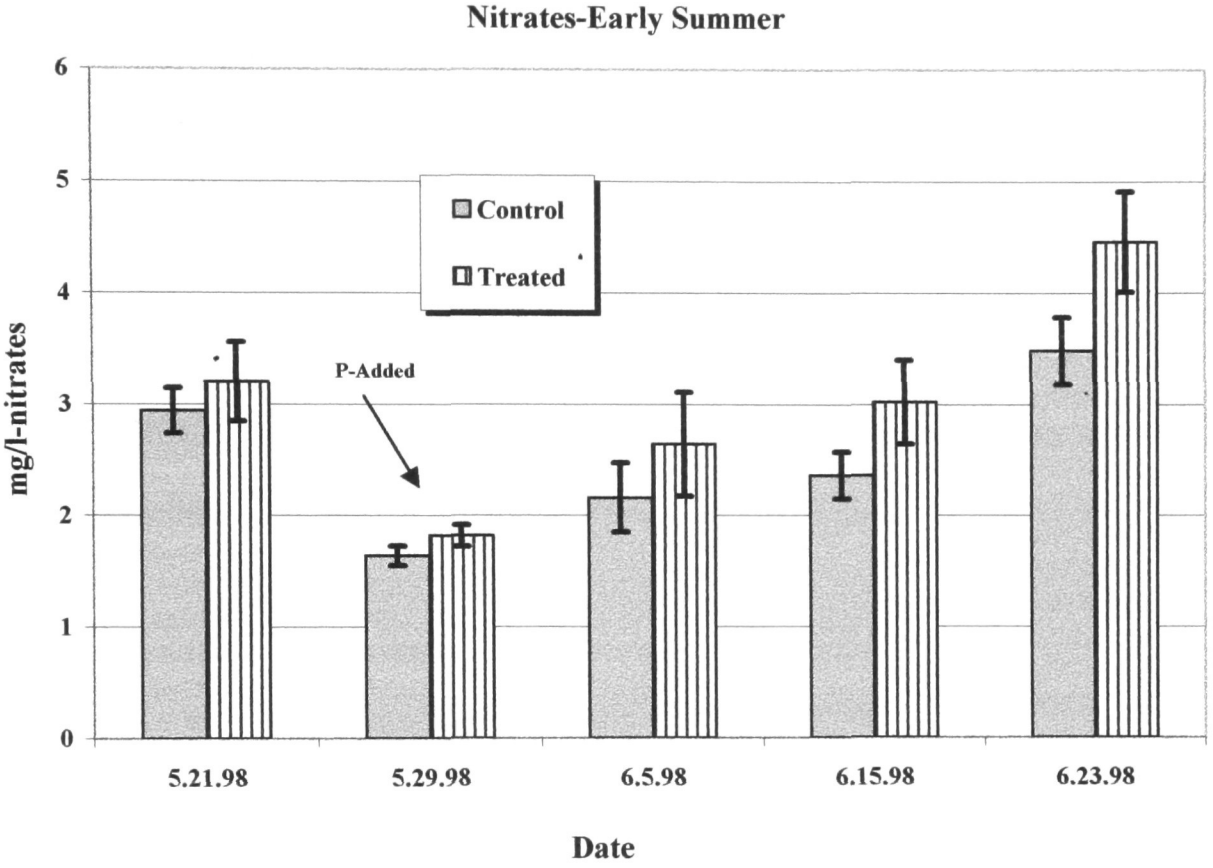

FIGURE 13: Mean nitrate (NOx) concentrations (mg/l)-Early summer experiment, May 21-June 23, 1998.

FIGURE 14: Mean chlorophyll *a* concentrations (micrograms/l)-Early summer, May 21-June 23, 1998.

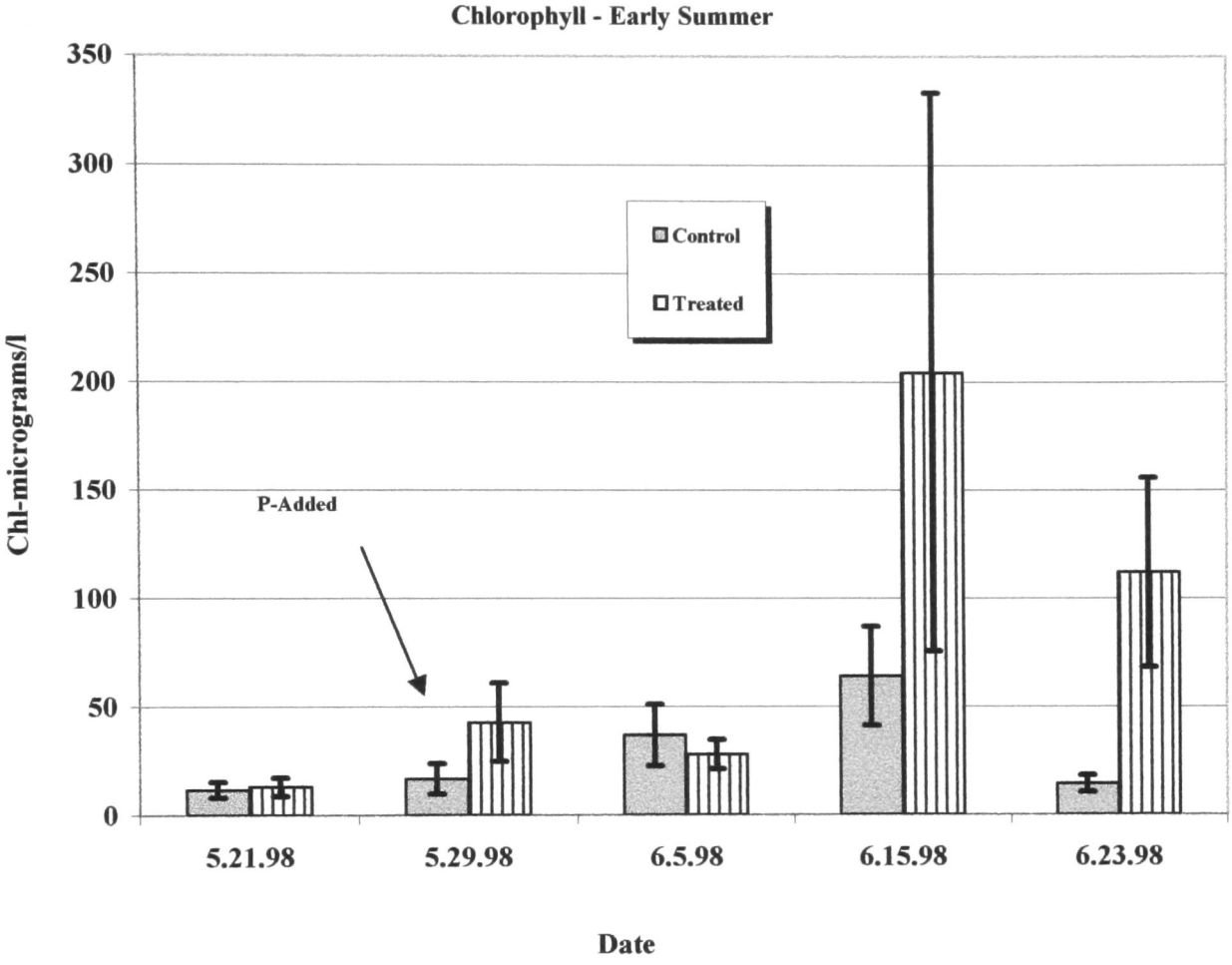

FIGURE 14: Mean Chlorophyll *a* concentrations (micrograms/l)-Early summer, May 21-June 23, 1998.

FIGURE 15: Mean temperature (Celsius)- Early summer, May 21-June 23, 1998.

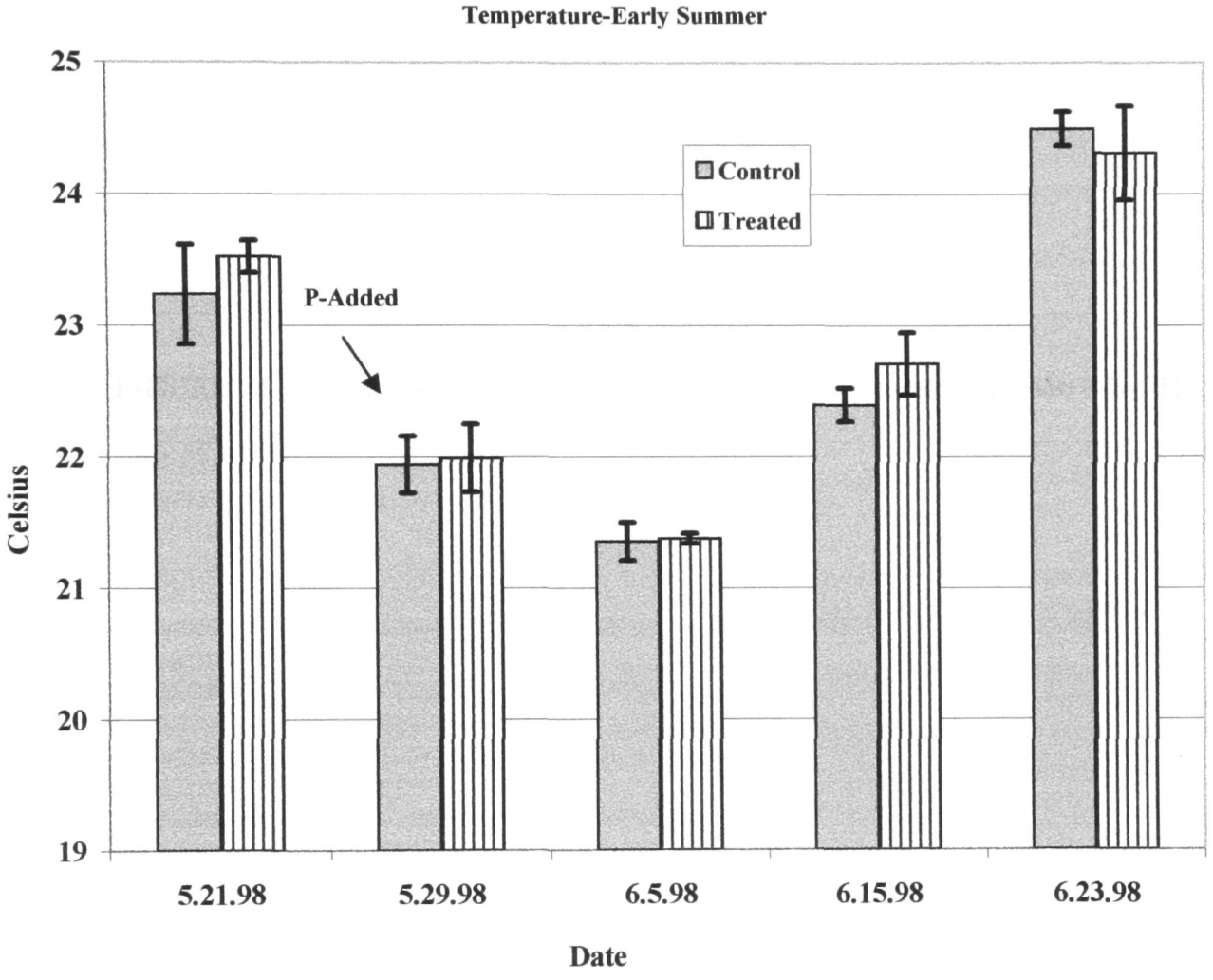

FIGURE 15: Mean Temperature (Celsius)-Early Summer, May 21-June 23, 1998.

FIGURE 16: Mean dissolved oxygen concentrations (mg/l)-Early summer, May 21-June 23, 1998.

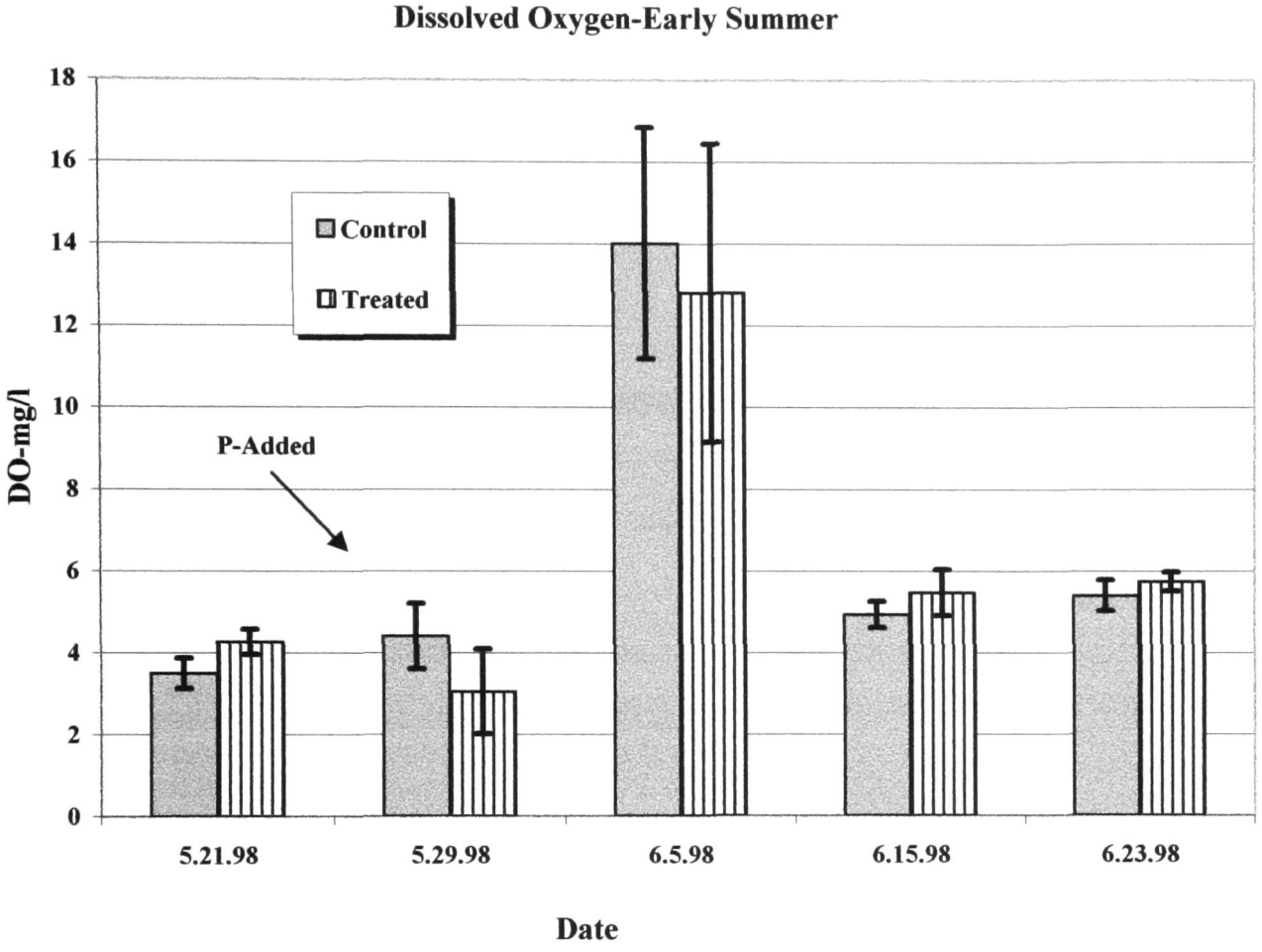

FIGURE 16: Mean dissolved oxygen concentrations (mg/l)-Early summer, May 21-June 23, 1998.

FIGURE 17: Mean turbidity (NTU)- Early summer, May 21-June 23, 1998.

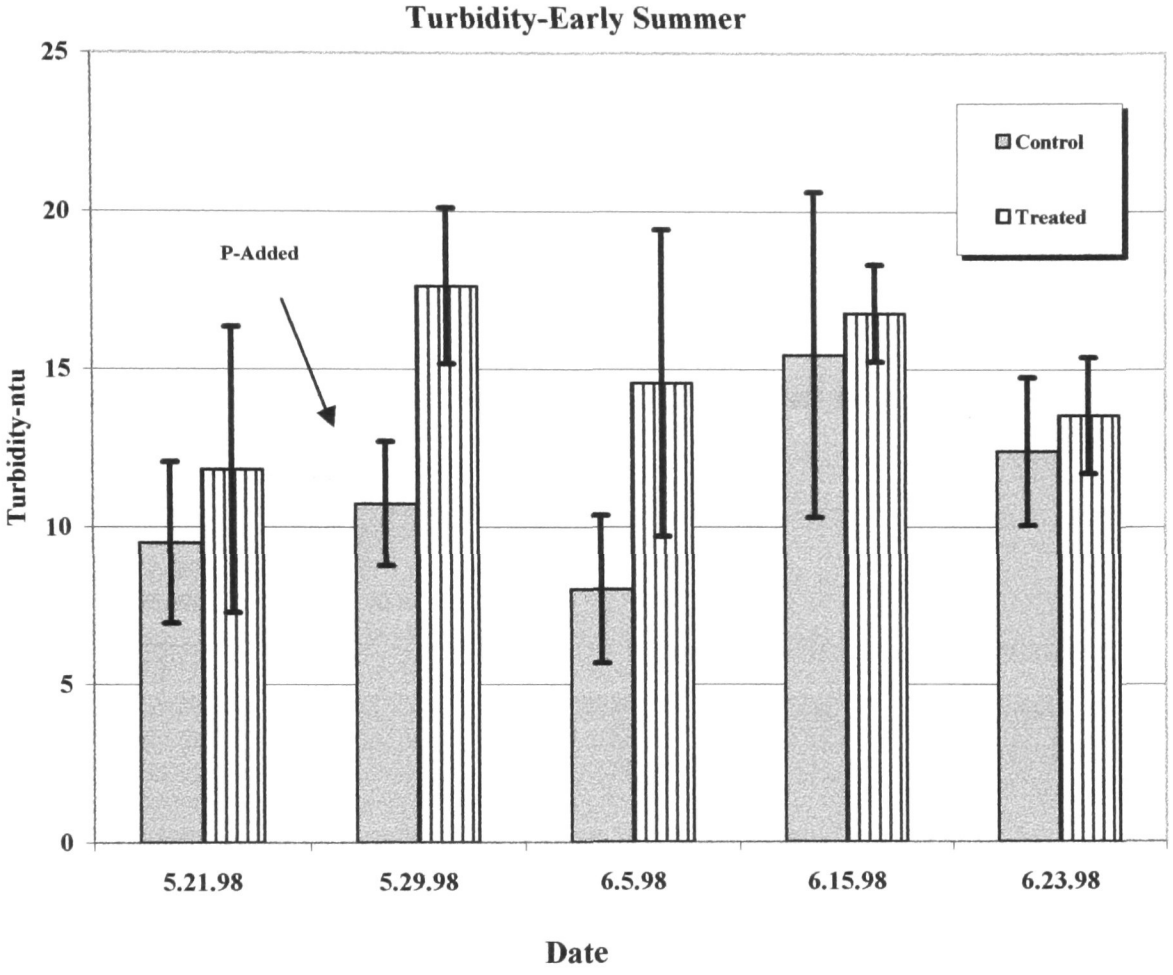

FIGURE 17: Mean turbidity (NTU)-Early summer
May 21-June 23, 1998.

FIGURE 18: Cyanophyceae-blue-green algae. Mean # organisms/0.2ml. Early summer- May 21-June 23, 1998.

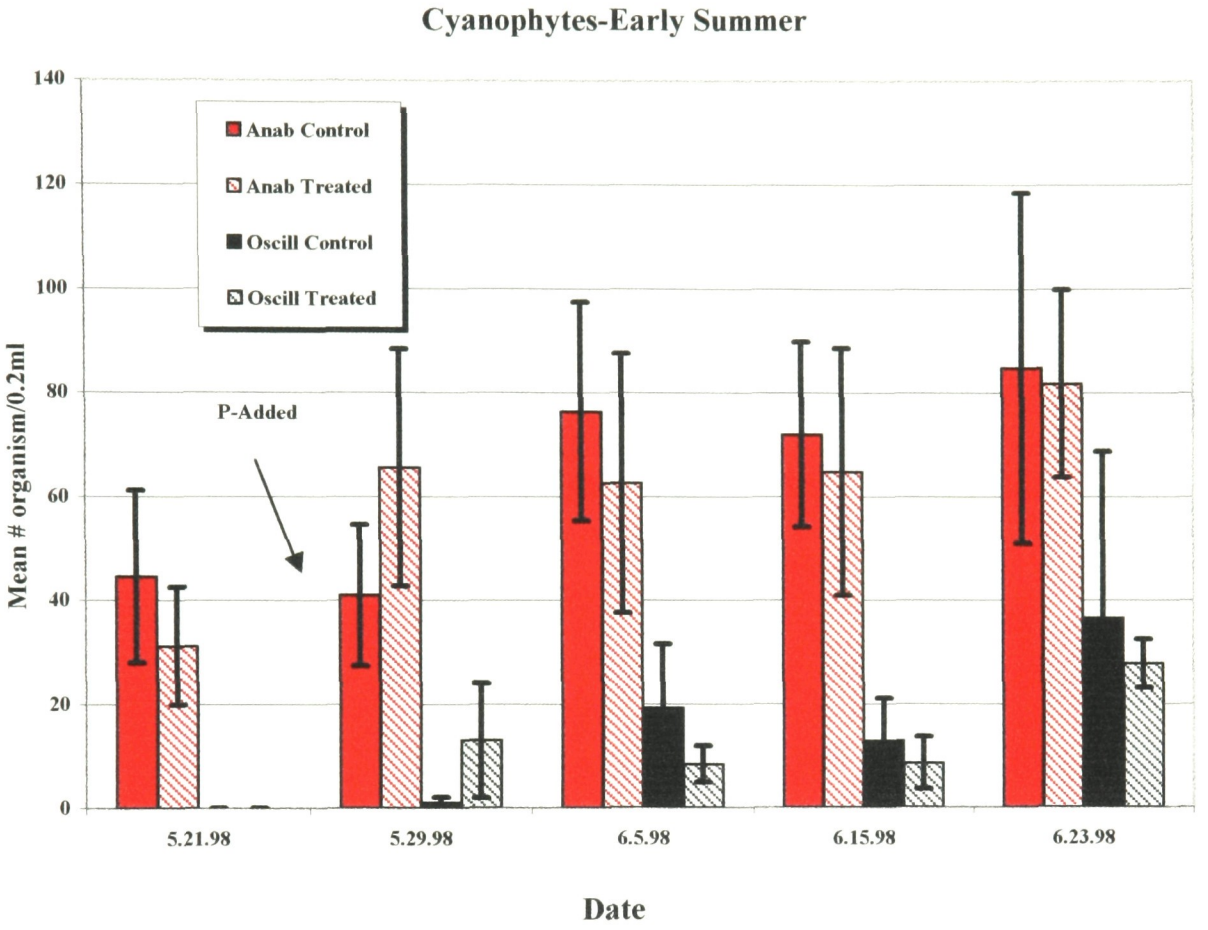

FIGURE 18: Cyanophyceae-blue-green algae. Mean # organisms/0.2ml. Early summer-May 21-June 23, 1998.

FIGURE 19: Chlorophyceae-green algae and Chrysophyceae-yellow-brown algae-Mean # organisms/0.2ml. Early summer, May 21-June 23, 1998.

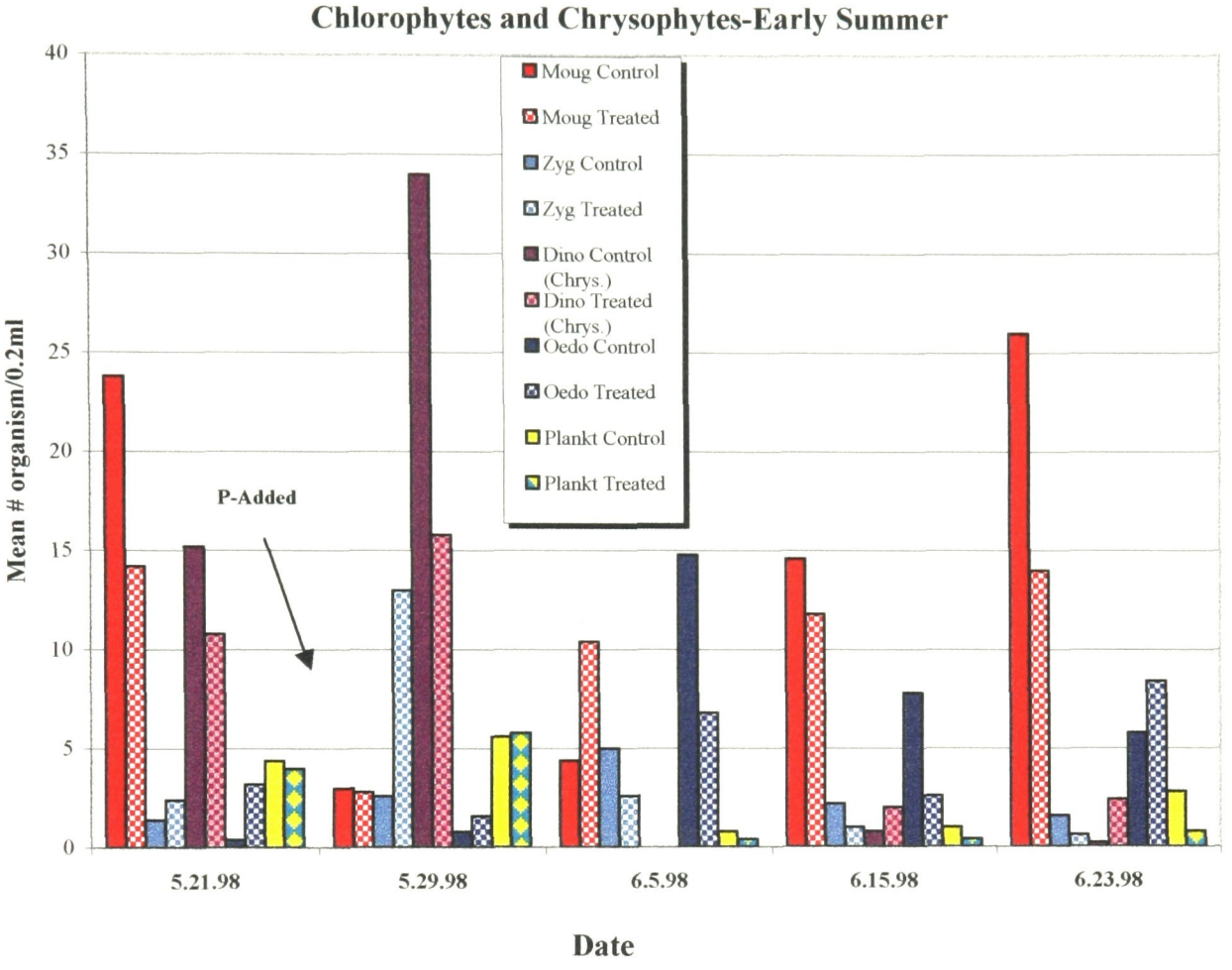

FIGURE 19: Chlorophyceae-green algae and Chrysophyceae-yellow-brown algae Mean # organisms/0.2ml. Early summer, May 21-June 23, 1998.

FIGURE 20: Bacillariophyceae-Diatoms. Mean # organisms/0.2ml. Early summer, May 21-June 23, 1998.

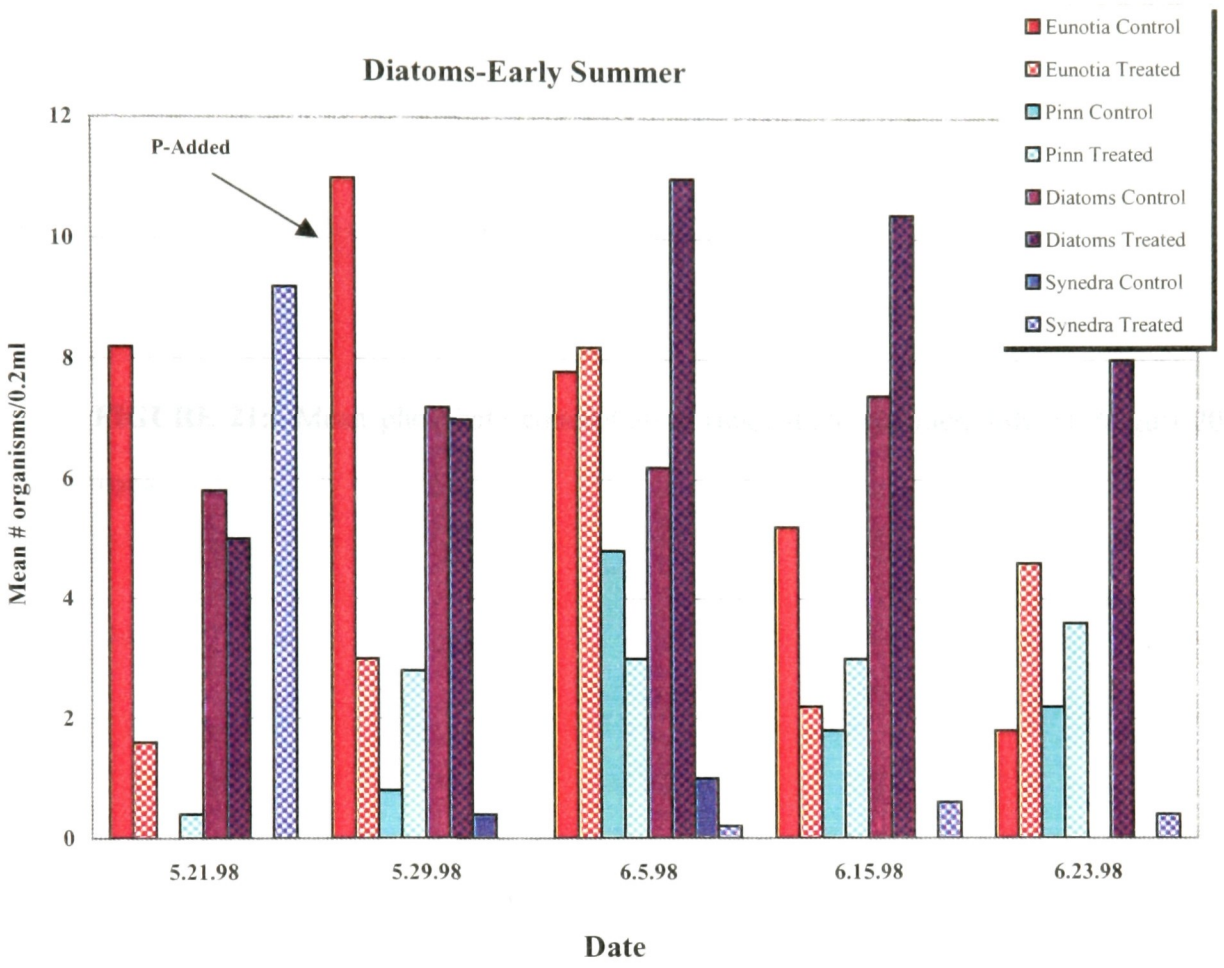

FIGURE 20: Bacillariophyceae-Diatoms. Mean # organisms/0.2ml. Early summer, May 21-June 23, 1998.

FIGURE 21: Mean phosphate concentration (mg/l)-Late summer, July 31-August 20, 1998.

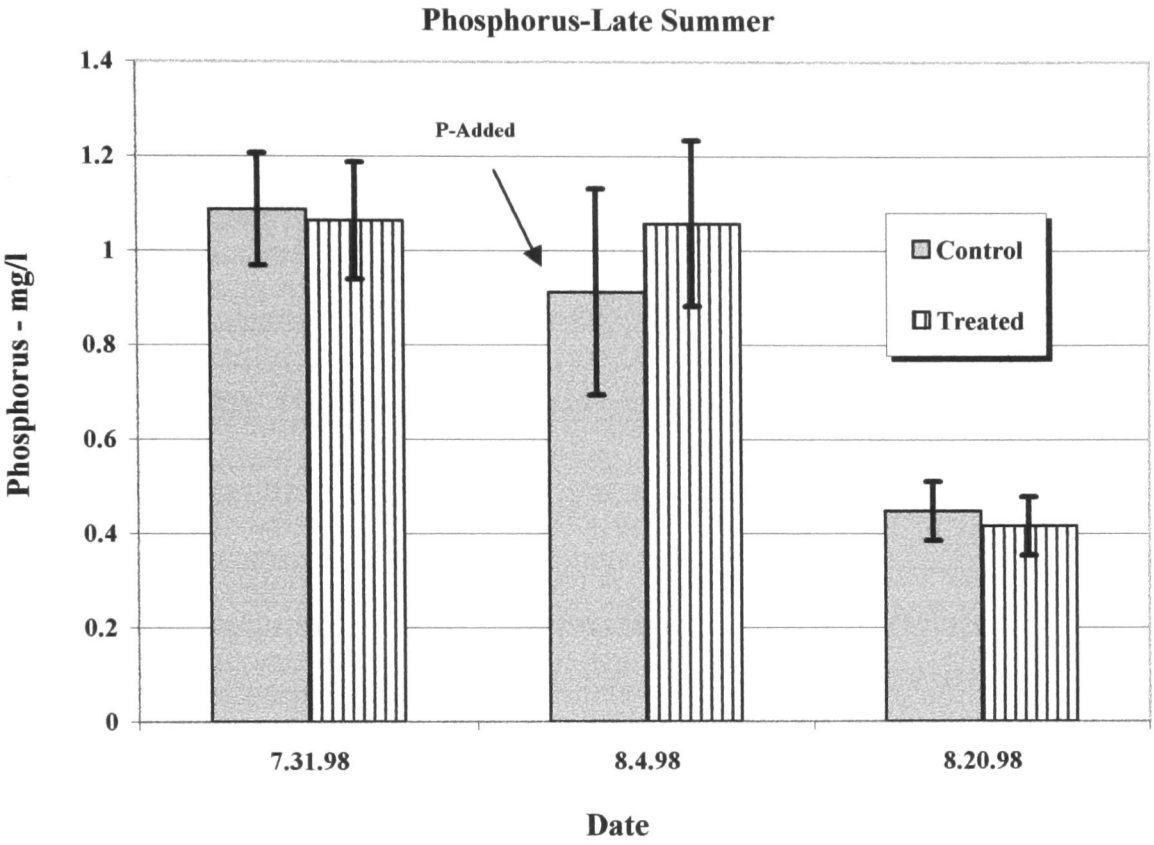

FIGURE 21: Mean phosphate concentration (mg/l)-Late summer, July 31-August 20,1998.

FIGURE 22: Mean nitrate (NOx) concentrations (mg/l)- Late summer, July 31-August 20, 1998.

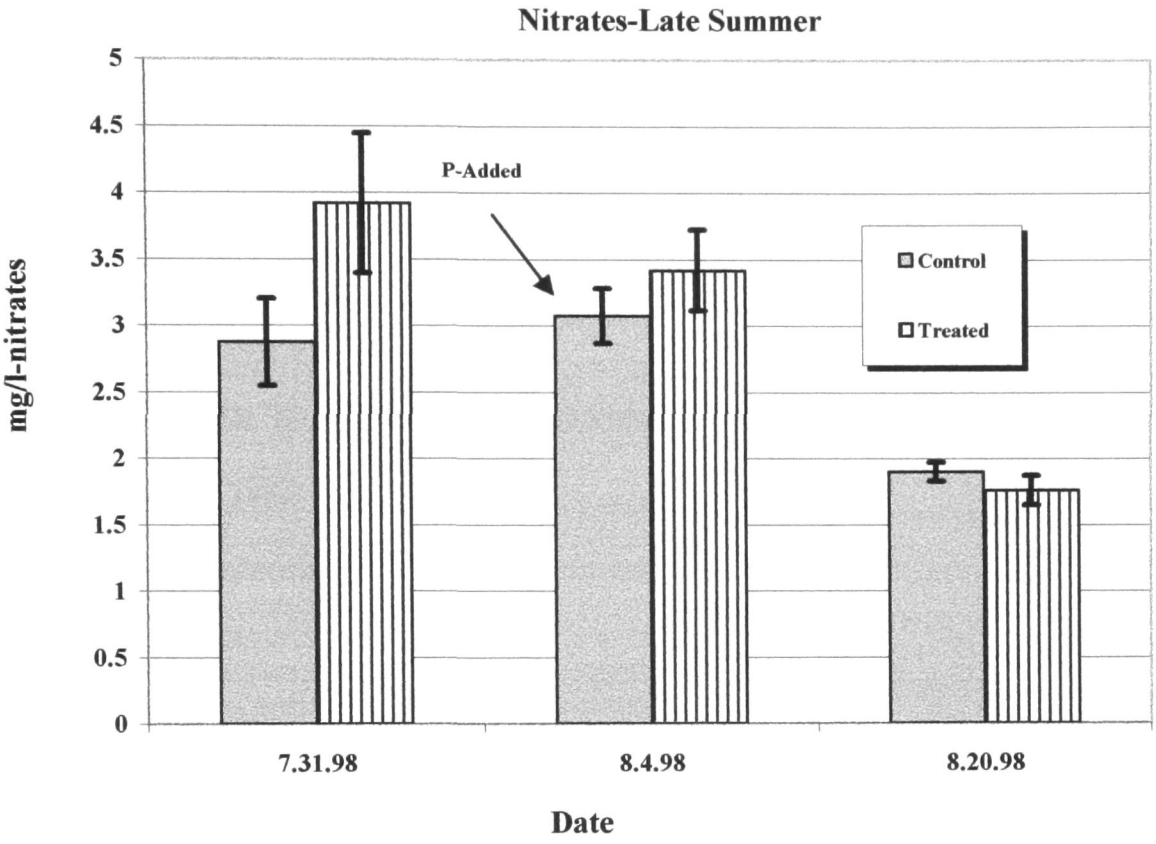

FIGURE 22: Mean nitrate (NOx) concentrations (mg/l)-Late summer, July 31-August 20, 1998.

FIGURE 23: Mean chlorophyll *a* concentrations (micrograms/l)- Late summer, July 31-August 20, 1998.

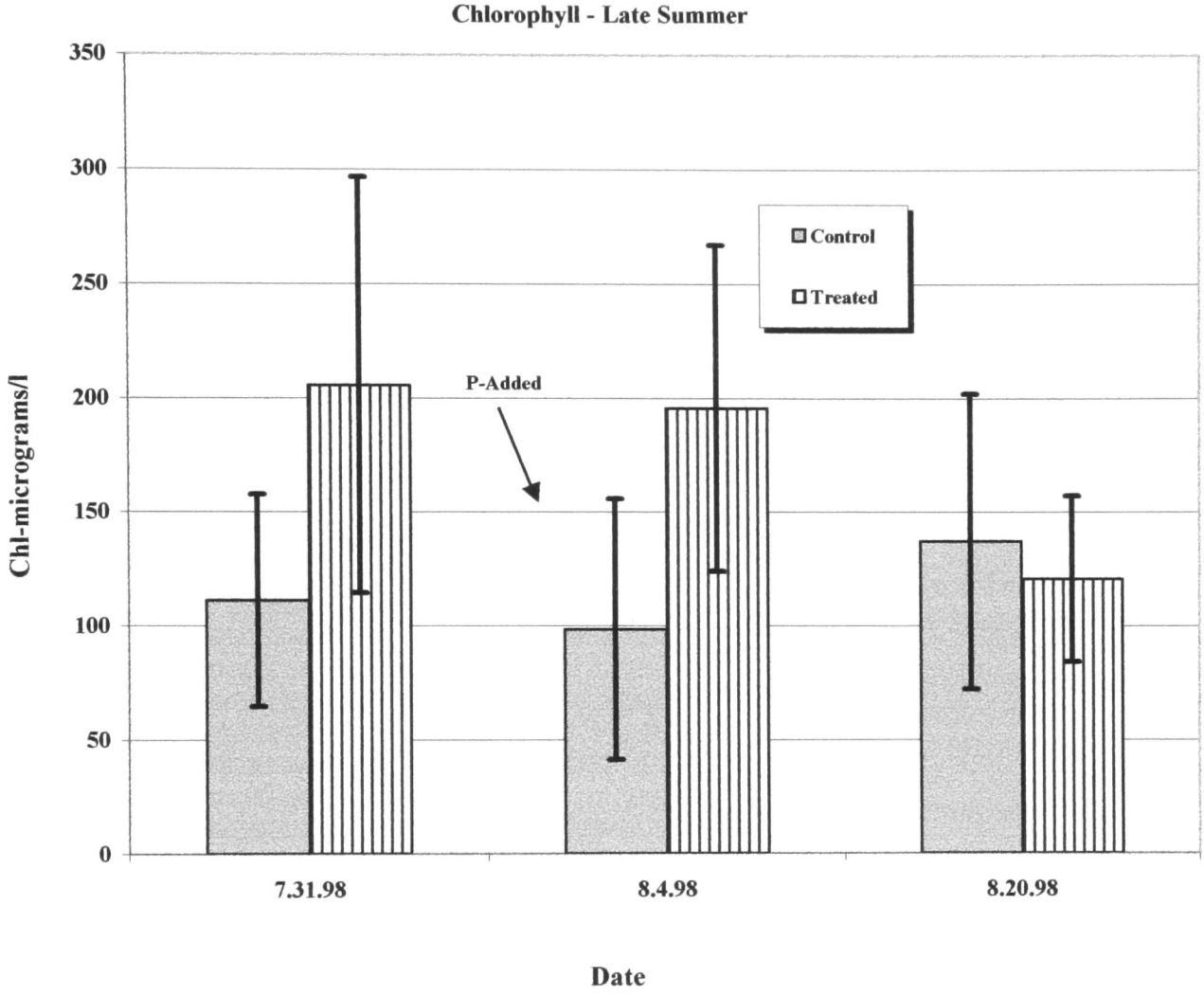

FIGURE 23: Mean chlorophyll *a* concentrations (micrograms/l)-Late summer July 31-August 20, 1998.

FIGURE 24: Mean turbidity (NTU)- Late summer, July 31-August 20, 1998.

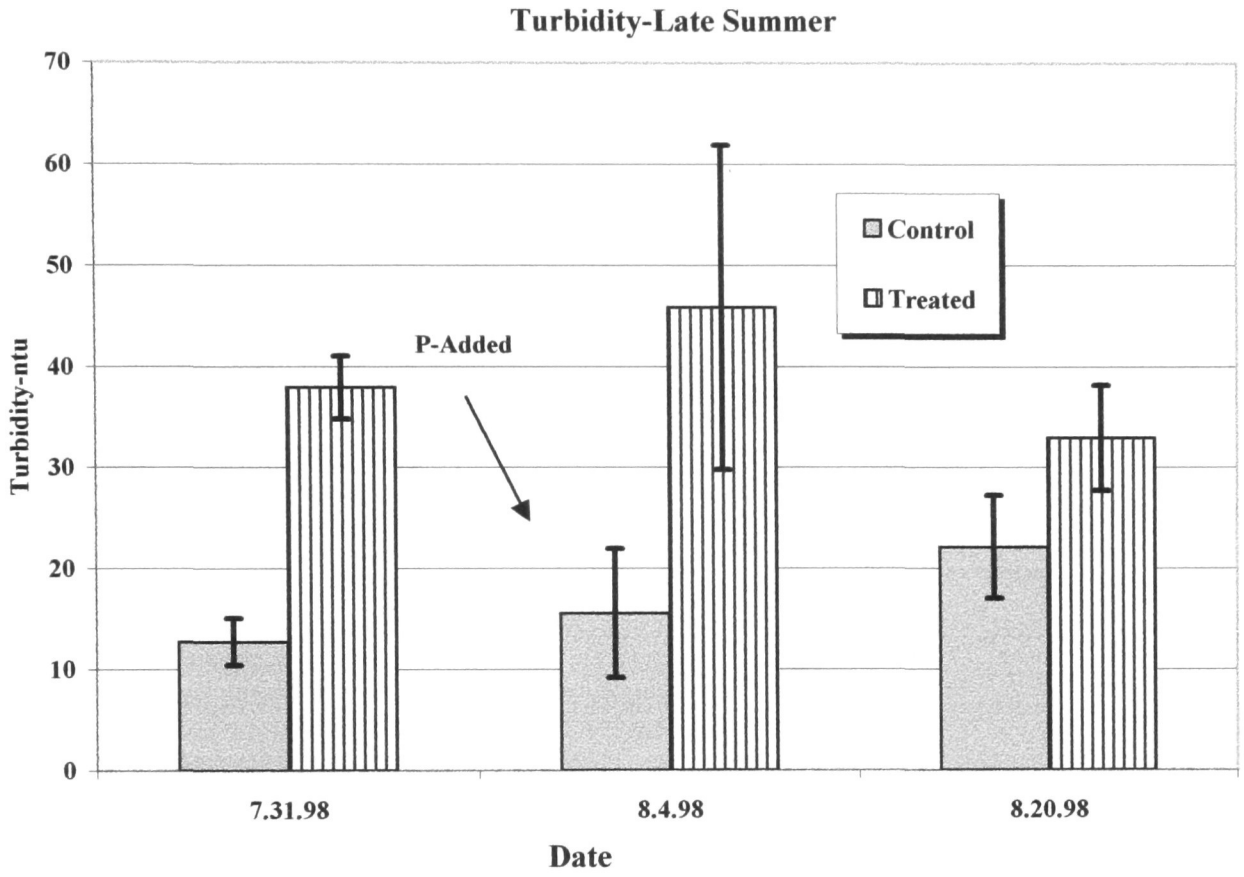

FIGURE 24: Mean turbidity (NTU)-Late summer, July 31-August 20,1998.

FIGURE 25: Cyanophyceae-blue-green algae. Mean # organisms/0.2ml. Late summer, July 31-August 20, 1998.

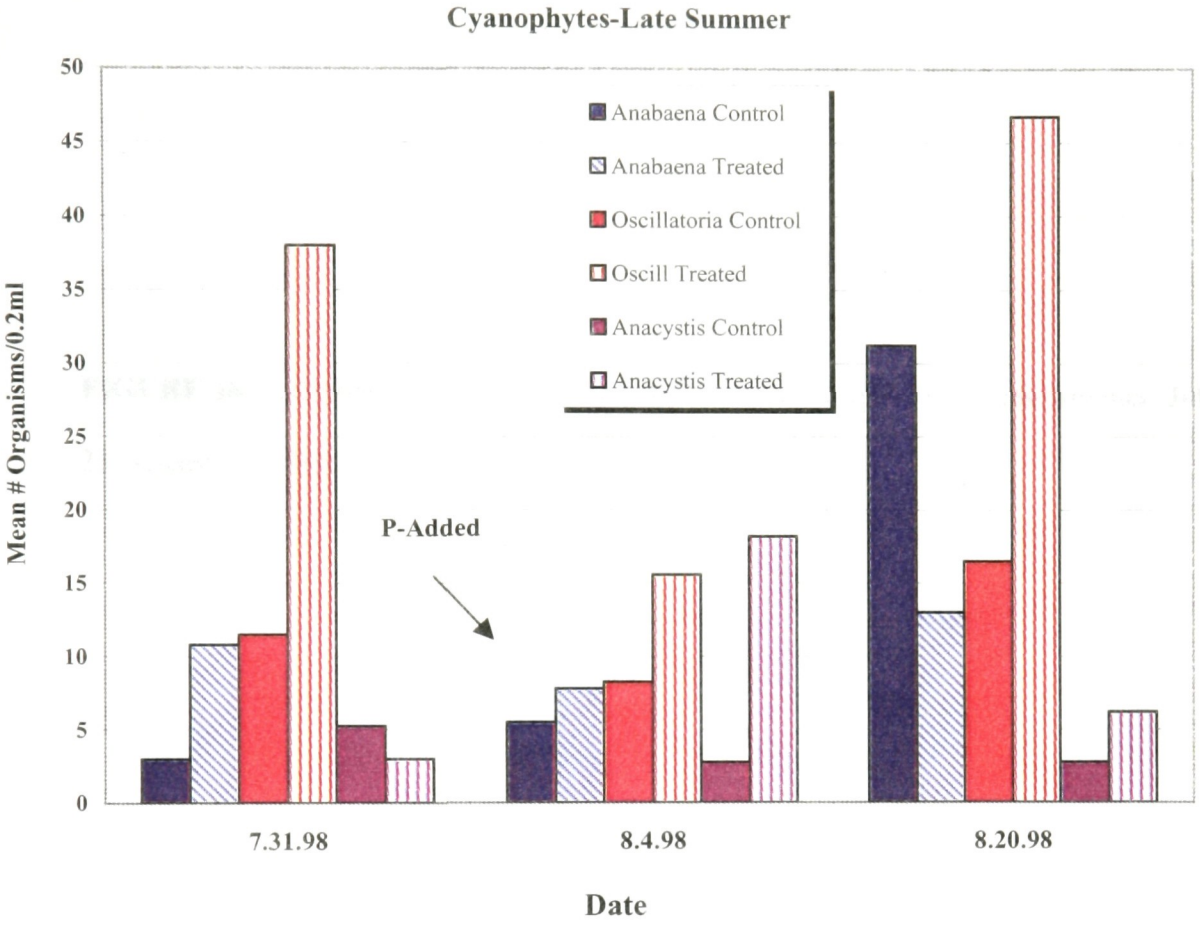

FIGURE 25: Cyanophyceae-blue-green algae. Mean # organisms/0.2ml. Late summer, July 31-August 20, 1998.

FIGURE 26: Chlorophyceae-green-algae. Mean # organisms/0.2ml. Late summer, July 31-August 20, 1998.

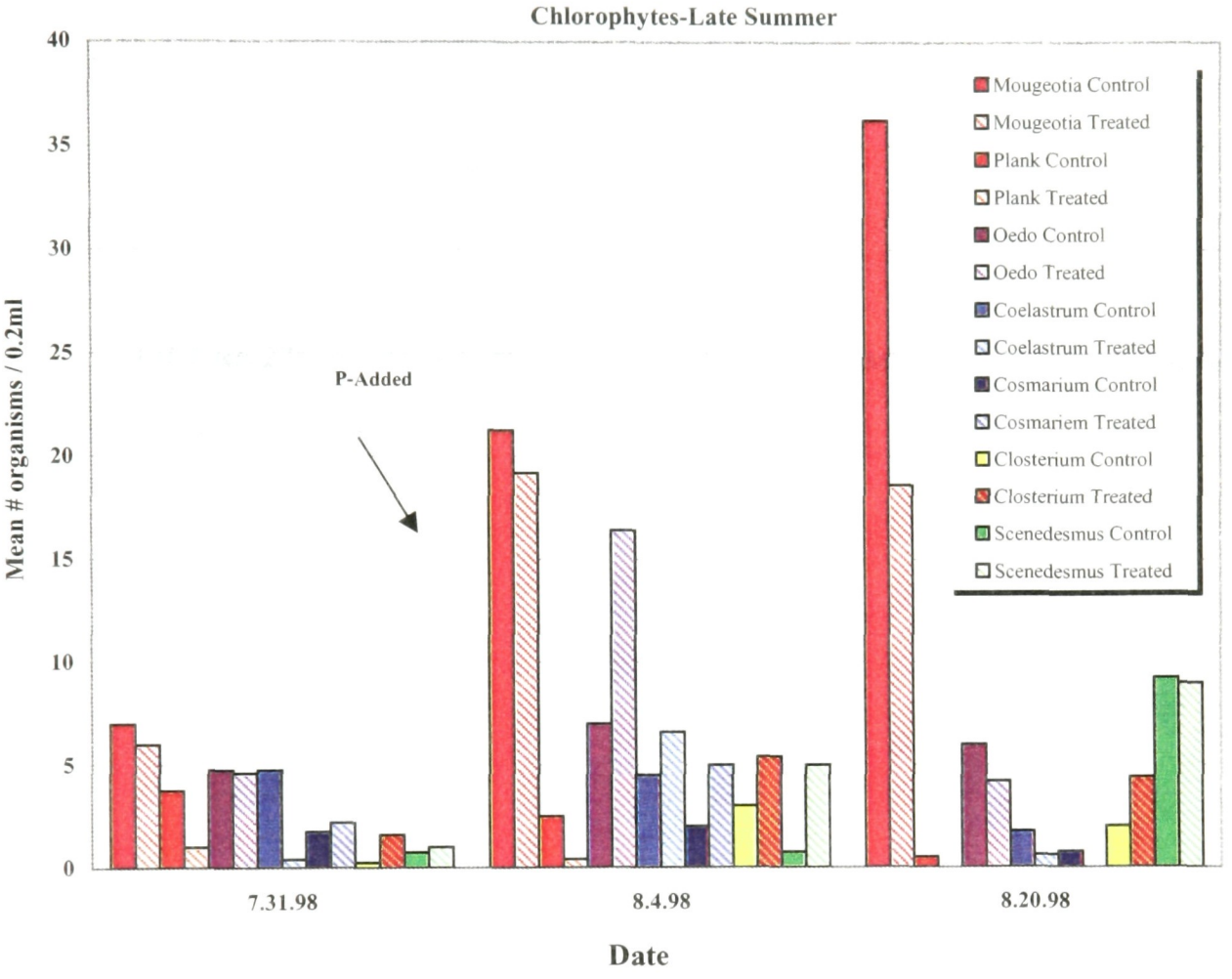

FIGURE 26: Chlorophyceae-green-algae. Mean # organisms/0.2ml. Late summer, July 31-August 20, 1998.

FIGURE 27: Bacillariophyceae-Diatoms. Mean # organisms/0.2ml. Late summer, July 31-August 20, 1998.

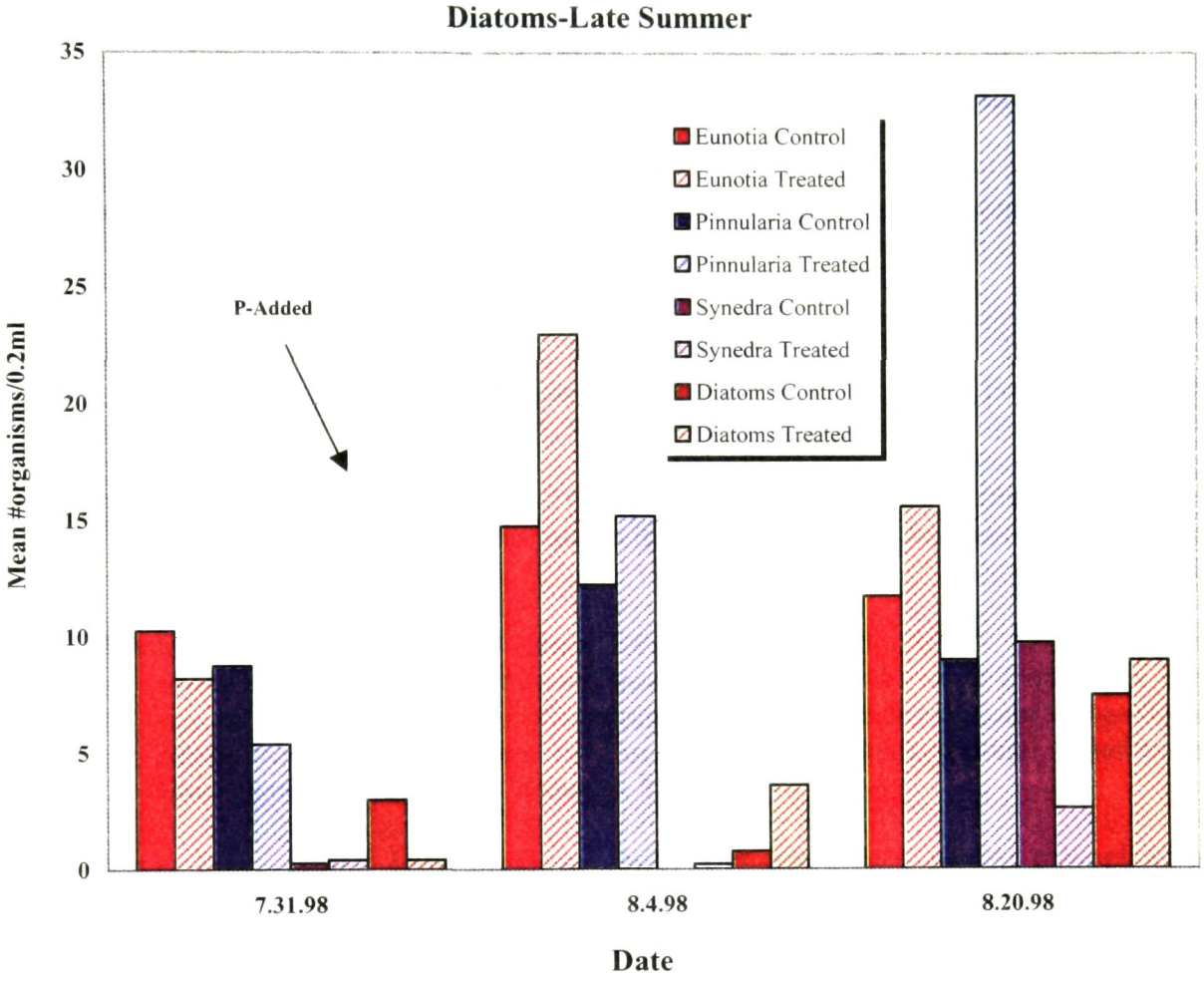

FIGURE 27: Bacillariophyceae-Diatoms. Mean # organisms/0.2ml. Late summer, July 31-Auguat 20, 1998.

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